Fermentation Volume Studies for Red Wine Experimentation

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Experimental vinification is often used to evaluate changes in viticultural and oenological practices in research trials. Microvinification procedures are used to overcome constraints that make standardised comparisons in commercial wineries difficult. Prior to 2009, a dedicated micro-winery research facility in northern Tasmania used conventional 12 L volume ferments that provided sufficient wine for both sensory and chemical analysis. Since then, much smaller ferment volumes of 1.5 L and of 250 mL have been introduced, and these provide a sufficient sample size for the chemical analysis of phenolic components in the wine. This study reports a comparison of the phenolic attributes of Pinot Noir wines in a replicated trial using must weights of 0.2, 1.0 and 10 kg fermented in vessels of volume 250 mL, 1.5 L and 20 L respectively. Using the same parcel of fruit, a single larger ferment of 330 kg and a vessel volume of 780 L was conducted concurrently. At bottling, six weeks after the end of fermentation, there was no significant difference in the phenolic composition of the wine made from grape musts with a mass of 0.2, 1.0 or 10 kilograms in the replicated trial, and the results were consistent with those for the 330 kg ferment size. We therefore have confidence in using small micro-scale fermenters, which greatly enhance research capability.

INTRODUCTION

Trials involving viticultural practices or winemaking methodologies require replication in order to conduct valid statistical analyses. Such conditions are difficult to achieve in commercial wineries due to the limitations imposed by production logistics, fermenter size and expense. Microvinification overcomes these problems by using small and uniform fruit volumes in temperature-controlled rooms, and has been used in Australia for over 40 years (Becker & Kerridge, 1972). Viticulture and oenology trials conducted in a 'micro-winery' research facility in Tasmania, Australia were initiated in 2005. Initially, red wine ferments were carried out in 20 L food-grade plastic buckets using 10 to 15 kg musts, producing sufficient wine for chemical analysis and sensory evaluation. Also, for many experiments, chemical analysis of the finished wine was sufficient to determine the outcome of the treatments applied. Subsequently, smaller must sizes of 700 g and 1 kg were conducted in 1.2 L cylindrical glass jars and 1.5 L Bodum® coffee plungers respectively, providing sufficient sample volumes for repeated phenolic analyses (Dambergs & Sparrow, 2011; Carew *et al*., 2013; 2014). These ferments were managed by using submerged caps, and chemical analysis showed very reproducible results. More recently, vessel size was further reduced (250 mL cylindrical polycarbonate jars with screw caps) for Pinot Noir wine trials (Smart *et al*., 2012; Sparrow *et al*., 2013). As little as 1.5 mL provides sufficient sample volume for phenolic analysis using ultraviolet-visible spectroscopy, as described by Dambergs *et al*. (2012b), calibrated against HPLC analysis (Sarneckis *et al*., 2006; Mercurio *et al*., 2007). This economical and demonstrably reproducible method of microvinification has been used to rapidly assess differences between parcels of fruit and the effects of winemaking additives and processes.

The extension of outcomes from small-scale winemaking trials to commercial practice can raise concern for some oenologists, for example because of the perceived influence of fermenting must temperature on wine quality parameters in response to the surface-to-volume ratio of the fermentation vessel (Hornsey, 2007). The current study compared the phenolic composition of newly bottled wines made from a homogenous parcel of fruit fermented under controlled temperature conditions at must weights of 0.2, 1.0 and 10 kg, and a single, larger ferment of must weight 330 kg conducted at ambient temperature.

MATERIALS AND METHODS

Grape sampling and replication

Grapes of *Vitis vinifera* cv. Pinot Noir clone D5V12, from

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drip-irrigated vines trained to vertical shoot positioning, were harvested from a 14-year-old vineyard in northern Tasmania (41.2°S; 146.9°E) in April 2013. Fruit from vines (yielding 8 t/ha) of two adjacent and uniform rows of 75 vines were hand harvested into a 0.5 tonne picking bin. Subsequently, a 60 kg sub-sample of grape bunches was taken at random from the picking bin and allocated to four 15 kg replicates. From each replicate, 200 g of randomly selected berries were frozen at -20°C and stored for grape colour and tannin analyses, and a further 100 berries were hand-crushed and the juice drained for fruit composition analyses. The remaining 330 kg of grapes were processed at the commercial winery on site.

Treatment preparation

For each replicate, grapes were de-stemmed and three ferment size treatments applied: (1) 200 grams of berries were crushed by hand in a sealed plastic bag and the contents transferred to a 250 mL polycarbonate jar; (2) 1 kg of berries was crushed by hand in a sealed plastic bag and placed in a 1.5 L Bodum® coffee plunger; (3) 12 kg of grapes were de-stemmed and crushed in a Marchisio Grape Crusher/De-stemmer (1 000 to 1 500 kg/h) and placed in a 20 L food-grade plastic bucket. The 330 kg of grapes were de-stemmed using a Bucher Vaslin Delta E4 Series Crusher/De-stemmer (25 to 30 t/h) at the commercial winery, and the must was placed in a 0.5 ton food-grade plastic fruit picking bin within the commercial winery.

Grape composition analysis

Total soluble solids in the grape juice (Brix) were measured using a refractometer, and the pH of the juice was measured using a Metrohm pH meter/autotitrator. Titratable acidity was determined by titration with 0.333 M NaOH to an end point of pH 8.2 and reported as grams/litre tartaric acid.

Grape colour and tannin analysis

Frozen whole berries were thawed overnight at 4°C and homogenised at 8 000 rpm for 20 seconds in a Retsch Grindomix GM200 homogeniser, with an S25 N-18G dispersing element (Janke & Kunkel GmbH & Co, Germany) fixed with a floating lid. One gram of homogenate was subsequently extracted in acidified 50% (v/v) ethanol for the determination of grape colour (Iland *et al*., 2004) and tannin (Dambergs *et al*., 2012b).

Vinification protocol

To each must, 50 mg/L SO_2 was added in the form of potassium metabisulphite. The smaller replicated must preparations (sizes 0.2 to 10 kg) were equilibrated to 25°C and inoculated with 300 mg/L RC212 yeast solution, before being fermented at 27°C (± 1°C). The largest must preparation (330 kg) was fermented at ambient temperature at the commercial winery. Inoculated at 17°C, the must temperature rose gradually to 23.5°C, where it was maintained until fermentation was completed. All fermentation vessels were covered with a loosely fitting lid and plunged twice daily. On day three of the fermentation, 300 mg/L of diammonium phosphate was added to each ferment. Eight days after inoculation, the ferments were confirmed 'dry', with less than 2 g/L of residual sugar detected using Clinitest® reagent tablets (Bayer Australia Ltd.). The two smaller ferment sizes (0.2 and 1 kg) were pressed by hand using a plunger with a 1 mm sieve fixed at the base, while the 10 kg and 330 kg ferments were pressed in a flat-bed press at 200 kPa of pressure. In each case, enough pressure was applied to recover 60% (v/w) of the must weight, that is 120 mL, 0.6 L, 6.0 L and 220 L of wine respectively. Wine was stored in glass screw-topped bottles at 4°C for 14 days, then racked under CO_2 cover and a further 80 mg/L of SO_2 was added. Following storage for a further 30 days at 12°C, the wine was racked prior to bottling, with a 10 mL sample taken for phenolic analysis.

Phenolic analysis by spectroscopy

Wine samples were clarified by centrifugation at 5 000 rpm for five minutes using an Eppendorf 5417C centrifuge with an Eppendorf F45-30-11 rotor (Crown Scientific, Australia). The phenolic composition of all samples was determined using the modification of the Somers assay, as described by Mercurio *et al*. (2007), and the rapid tannin assay (Dambergs *et al*., 2012b).

The phenolic parameters quantified were total tannin (pigmented and non-pigmented tannins); total phenolics (coloured and non-coloured tannins and anthocyanins, and low molecular weight, non-pigmented phenolic compounds); free anthocyanin (unbound anthocyanins); non-bleachable pigments (resistant to bleaching in the presence of sulphur dioxide, consisting of either pigmented tannins in which one or more anthocyanin molecules have become bound to proanthocyanidins (condensed tannins) (Harbertson *et al*., 2003) or pyranoanthocyanins (Boulton, 2001; Cheynier *et al*., 2006); colour density (the degree of pigment saturation of the wine); and hue (the nature of wine colour – higher hue values appearing ruby-garnet, and lower values appearing more blue-purple). UV-visible spectrophotometric analysis was conducted using a Thermo Genesys™ 10S UV-Vis Spectrophotometer. Samples were scanned in 10 mm cuvettes at 2 nm intervals over the wavelength range 200 to 600 nm.

Statistical analyses

Statistical analyses were conducted using GenStat 64-bit Release 14.2 Copyright 2011, VSN International Ltd. The mean and standard deviations for fruit composition characters, the phenolic content of grapes and the individual phenolic analytes in wine samples for each treatment replicate (n = 4) were calculated using one-way ANOVA. The 330 kg ferment was unreplicated and excluded from statistical analyses.

RESULTS

Fruit composition

Grape composition at harvest was 21.2 ± 0.5°Brix, pH 3.22 ± 0.04 and titratable acidity 7.70 ± 0.2 g/L. The total phenolic composition of the grapes consisted of 0.6 ± 0.03 mg/g anthocyanin and 6.9 ± 0.2 mg/g tannin, which is in accordance with reported values for Pinot Noir (Cortell & Kennedy, 2006; Cortell *et al*., 2007; Kemp *et al*., 2011).

Phenolic composition at bottling

For each of the phenolic analytes assessed at bottling, total phenolics, tannin, colour density, total pigment, free anthocyanin, non-bleachable pigment (resistant to SO_2 bleaching) and percentage non-bleachable pigment values were consistent and were not correlated with either the must weight or the surface area-to-volume ratio of the fermenting musts (0.2 kg, 1 kg, 10 kg musts or 330 kg). The surface-to-volume ratio (SA:V) of the fermenting must became smaller as the fermentation vessel size increased (Table 1). There was a slight tendency for hue to be less with the larger vessel sizes (Table 2). However, data from the single larger ferment vessel did not follow this trend.

DISCUSSION

To our knowledge, trials to determine the effect of fermentation volumes on the phenolic composition of wine grapes has not been reported previously. A variety of changes in either viticultural or oenological practices have been evaluated in our laboratory using the smaller vessel volumes described here. These include yeast strain effects on wine quality, maceration techniques during vinification, and wine quality responses to UV radiation and vine vigour (Carew *et al*., 2013; Dambergs *et al*., 2012a; Carew *et al*., 2013; Song *et al*., 2014; 2015).

This study demonstrates that, for seven out of the eight phenolic characters assessed in wines made from Pinot Noir grapes, the phenolic composition at bottling was independent of fermentation vessel (must) size over the weight range 0.2 to 10 kg.

The larger ferment size (330 kg), conducted concurrently using the same fruit source, did not have the same strict conditions of fermentation temperature. However the values for each phenolic parameter in the wines from this ferment size were within the range of those of the three smaller ferments (Table 2). This suggests that the results of the microvinification trial may be extrapolated to larger ferment sizes, but such a conclusion warrants further controlled comparisons. While this investigation focused on the phenolic composition of Pinot Noir wine, potential differences in aroma and flavour attributes of wines made in fermentation vessels of diverse size and surface area-to-volume ratio are also worthy of further investigation.

To maintain similarity with the larger ferment in this study, all smaller volume ferments were plunged twice daily, whereas in practice we found submerged cap fermentation to be well suited to the micro-fermentation procedure. When using fermentation vessels of small volume, experiments compromising 20 to 80 plots could be conducted routinely at low cost and with reduced labour input, thus greatly increasing our research output.

CONCLUSIONS

This study confirmed the appropriateness of microvinification as a tool that facilitates research in that many more treatments and replicates can be handled easily. It also demonstrated

TABLE 1

Must sizes for ferments made in vessels of different capacity.

Vessel capacity (L)	0.25	1.5	20	780
Must weight (kg)	0.2	1	10	330
Must diameter (cm)	6.5	12	26	NA
Must height (cm)	6.5	11.3	24	31
Must surface area (cm^2)	33	113	531	10 000
Must volume (L)	0.22	1.28	12.7	310
Must surface area:volume	0.15	0.09	0.04	0.03

NA, not applicable

TABLE 2

Phenolic composition, at bottling, of Pinot Noir wine made from fermentation vessels of varying must capacity. Data for replicated ferments are mean and standard deviation (n = 4).

Must size (kg)	0.2	1	10	[a]330	*P-value*
surface area:volume ratio	0.15	0.09	0.04	0.03	
Tannin (g/L)	0.23 ± 0.07	0.21 ± 0.07	0.33 ± 0.14	0.22	0.83
Anthocyanin (g/L)	0.16 ± 0.01	0.17 ± 0.02	0.20 ± 0.03	0.15	0.30
NB pigment (AU)	0.33 ± 0.05	0.32 ± 0.03	0.32 ± 0.06	0.30	0.94
Total pigment (AU)	8.54 ± 0.76	8.86 ± 1.08	9.88 ± 1.78	7.91	0.14
% NB pigment	3.82 ± 0.34	3.65 ± 0.22	3.23 ± 0.40	3.80	0.06
Total phenolics (AU)	24.6 ± 1.33	24.3 ± 1.96	28.1 ± 3.44	24.0	0.06
Colour density (AU)	2.67 ± 0.34	2.67 ± 0.25	2.80 ± 0.36	2.46	0.21
Hue (AU)	0.73 ± 0.02 a	0.71 ± 0.02 ab	0.68 ± 0.01 b	0.81	0.04

NB = non-bleachable pigment; AU = absorbance units. For each phenolic parameter, only means followed by a different letter were significantly different using Tukey's test ($p \leq 0.05$). [a] Unreplicated and excluded from statistical analyses.

that the phenolic evaluation of wine is independent of vessel volume over the must weight range from 200 grams to 10 kilograms, and may be representative of larger vessels up to 330 kg.

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