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Evaluation of Red Wine Made on a Small Scale Utilizing Frozen Must

FRANK SCHMID,† YAN LI,‡ BRIONY LIEBICH,# JULIE CULBERT,# CHRIS DAY,# AND VLADIMIR JIRANEK*,†

School of Agriculture, Food and Wine, The University of Adelaide, PMB 1, Glen Osmond, South Australia 5064, Australia, Alcoholic Beverage Laboratory, College of Bioscience & Bioengineering, Hebei University of Science & Technology, 186 Yuhua East Road, Shijiazhuang 050018, China, and Provisor Pty Ltd, P.O. Box 243, Glen Osmond, South Australia 5064, Australia

This paper describes the use of frozen-stored red must as an alternative to fresh must to permit research fermentations outside the vintage period. Additionally, the fermentation size (20, 50, and 300 kg) was also compared. Chemical analyses of six wines showed little variation in color profiles and final ethanol and organic acid concentrations. More importantly from a winemaking point of view, a descriptive sensory analysis revealed that all wines across each treatment and fermentation scale compared very well to each other. Key differences were limited to more appealing characteristics (i.e., lower tannin hardness and burnt/smoky attributes and higher fresh/fruity and red berry attributes) in the wine made on a 300 kg scale from frozen must. This study therefore provides quantitative data on the effectiveness of freezing for fruit preservation as well as the ability of small volume fermentations (20 and 50 kg) to be representative of conditions approaching those found in industry.

KEYWORDS: Frozen must; red wine; Cabernet; fermentation size; descriptive sensory analysis

INTRODUCTION

White wines are produced from the juice of white or red grapes, whereas red wine production dictates the extended maceration of grape skins in the fermenting liquid so as to extract pigments into the wine (*1*, *2*). To study red winemaking, it is preferable to utilize fresh fruit, which is prepared and fermented in a configuration reflective of the industrial situation. This dependence on fresh fruit largely restricts oenological research, whether academic or industry based, to the vintage period. To work outside of this window, model grape systems or else appropriate means of storing and preserving grapes are required. Preservation by freezing, as commonly practiced in the food industry, may be a possible means of overcoming the seasonal limitation on fruit availability. Yet the use of frozen whole grapes or must to make wine has received limited attention despite anecdotal claims that wine made from frozen musts/grapes can be of equal quality to that made from fresh grapes.

Some evidence suggests that grapes, frozen and thawed, quickly develop undesirable flavor and color characteristics (*3*). However, Brown (*4*) showed that these problems could be overcome by addition of antioxidants such as SO_2 directly into the crushed grapes. Sulfur dioxide also proved beneficial for storage of grapes in coated cellophane bags at 0 °C (*5*). In comparison, the quality of wine made from frozen whole grapes was not adversely affected (*6*, *7*). Chemical differences in wines made from frozen compared to fresh grapes included higher potassium concentrations, lower phenol and caffeoyl tartrate concentrations, but similar color profiles (*7*). A more recent study by Cynkar et al. (*8*) showed that berries with high initial color stored whole at −18 °C for 6 months contained only between 5 and 10% less total anthocyanins than the fresh fruit. Although it seems that the common problems associated with freezing grapes/musts for subsequent fermentation can be overcome, it must be remembered that the treatments discussed above were generally evaluated on trials of only 0.5−2.0 kg, and never more than 10 kg. Also, Pinot Noir and black muscadine grapes are the only red varieties examined so far, and any chemical or sensory analysis of resulting wines has been limited.

We therefore conducted small-scale red winemaking trials comparing wines made from fresh must (control wine) to frozen must to more fully define the consequences for wine composition and quality of this preservation treatment. In keeping with the intended application of this approach to the fermentation volumes more commonly used in research winemaking, trials were carried out with >10 kg aliquots of fruit. In fact, to gauge the influence of fermentation size on our findings, fermentations were performed in three batch sizes: 20, 50, and 300 kg. The larger fermentation system is equivalent to the small commercial

* Corresponding author. Fax: +61 8 8303 7415. Phone: +61 8 8303 6651. E-mail: vladimir.jiranek@adelaide.edu.au.
† The University of Adelaide.
‡ Hebei University of Science & Technology.
# Provisor Pty Ltd.

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scale, as is widespread in Europe, and is increasingly being accepted as representative of larger scale systems (*9*−*13*).

MATERIALS AND METHODS

Grape Processing and Storage. Cabernet Franc and Cabernet Sauvignon grapes (chosen due to availability) were hand harvested in late April 2005 from a private vineyard near Ashton, Adelaide Hills, South Australia, and stored at 0 °C overnight. Bunches were randomly sorted by weight to produce an approximate Cabernet Franc/Cabernet Sauvignon blend of 1:2 before crushing/destemming and allocation into triplicate batches. A Gamma 50CE-RM (Toscana Enologica Mori, Italy) crusher and destemmer was used for the 300 kg batches, and an Enoitalia ENO-15 (Winery Supplies, Australia) unit was used for the 50 and 20 kg batches. PMS (50 mg/L) was added to all musts after processing. Musts for both trials were immediately transferred into their respective fermentation vessels (see below); those representing the control were held at 0 °C before initiation of fermentation, whereas those for the frozen must trial were sealed and stored at −18 °C. We deliberately chose to freeze musts rather than whole bunches so as to avoid additional handling of grape bunches (e.g., the removal of frozen grapes from stems) and to avoid differences between frozen vs control treatments related to differential extraction of material from stems. Initiation of the fermentations was staggered due to lack of fermentation space with frozen musts being inoculated 26 days after the control musts.

Fermentation of Control and Frozen Musts. All fermentations were carried out following the standard winemaking practices used in the Hickinbotham Roseworthy Wine Science Laboratory. Prior to inoculation, frozen musts were thawed at 0 °C (approximately 48 h), and then all musts were brought to 20 °C overnight in a 20 °C room. Standard additions of tartaric acid (2.4 g/L) and diammonium phosphate (150 mg/L) were made to the musts before inoculation. Dry yeast (PDM, Maurivin), used at a rate of 20 g/hL, was reconstituted and added to the must following the manufacturer's instructions. Rotary fermentors (900 L capacity, A & G Industries, Australia; see ref *14*)) were used to ferment the 300 kg lots, and sealable plastic containers were used to ferment both the 50 and the 20 kg lots. All 20 and 50 kg ferments were held in a 20 °C temperature controlled room, whereas 300 kg ferments were carried out at ambient temperature (average temperatures over the course of fermentation were 18.4 ± 0.4 °C for control musts and 16.3 ± 1.4 °C for frozen stored musts) as temperature control of the rotary fermentors was limited to cooling only. Samples were taken from each of the smaller fermentations after the cap was manually plunged every 12 h (10 and 15 times total for the 20 and 50 kg ferments, respectively). Rotary fermentors were turned 3 times clockwise and 3 times counterclockwise every 12 h. Once below 4° Bé the wines were pressed as soon as equipment became available. The 300 kg batches were pressed using a Willmes VP1800 press (two presses at 1 bar, two presses at 2 bar, with two crumples between presses), into 280 L holding tanks and kept at 20 °C. The 50 and 20 kg batches were pressed twice using a basket press (SIRIO 60, Costruzioni Enologiche Padovane, Italy) operated at up to 2 bar with a rummage in between presses, and the wine was transferred to appropriately sized demijohns 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 manufacturer's instructions and underwent malolactic fermentation (residual malic acid concentration >0.05 g/L by enzymatic test kit, Roche catalog no. 10139068035). Prior to bottling, wine pH was adjusted to 3.55 using a tartaric acid solution (20% w/v) and stabilized by the addition of potassium hydrogen tartrate (4 g/L). Total SO_2 was adjusted to 75 mg/L. The bottled wines were stored at 15 °C in cellar conditions for at least 1 month prior to sensory evaluation.

Color Analysis. Must and wine samples were analyzed for both total red pigments and total phenolics following the method of Iland et al. (*15*) modified to allow spectral measurement using 96-well UV-transparent microtiter plates (Corning). Samples (50 μL) 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 microtiter plates. Absorbances (520 and 280 nm) were determined using a plate reader (μQuant Microplate spectrophotometer, Bio-Tek Instruments) set to automatic path length correction. Total red pigments were estimated under the assumption that all were present in the malvidin-3-glucose form with an extinction coefficient of 500 (*15*). Total phenolics were quantified against a gallic acid standard curve (0.0−0.4 mg/mL) and expressed as gallic acid equivalents (GAE).

Table 1. Sensory Attributes with Definitions and Composition of Reference Standards (Where Used) for the Descriptive Analysis of Red Wines

attribute	definition
	Aroma
freshness	impression of clean, fresh aromas that are not dull
red berry	fresh raspberry, strawberry
dark berry	fresh blackberry, black cherry, plum
dried fruit	includes raisin, stewed/cooked fruit
confection	includes raspberry lolly, bubblegum
stalky	includes green, herbaceous, leafy or stem-like characters
vegetal	includes capsicum and cooked beans
mint	minty, menthol character
sweet spice	mixed spice, cinnamon, cloves, like fruitcake spices
chocolate	includes dark chocolate and chocolate flavored lollies[a]
earthy/musty	includes dull, damp cellar, wet paper/cardboard
burnt/smoky	includes any reductive, burnt rubbery character
volatile acidity	includes ethyl acetate, vinegar-like aromas
	Palate
acidity	degree of sourness, a tart, sharp taste
ripe berry	intensity of ripe berry/cherry fruit, sweet jammy fruit
stalky/unripe	intensity of green, unripe flavors of fruit not fully ripened
body	fullness, how heavily flavors are perceived, low = light, high = heavy/full
viscosity	consistency of liquid, low = watery/thin, high = heavy/full
drying	astringent, puckering feeling around mouth and tooth surfaces
tannin hardness	degree of harshness of tannins, low = soft, smooth, high = harsh, extracted
bitterness	a taste like caffeine, noticed at the back of the throat
metallic	a hard finish and flavor of metal or like blood
length	persistence of fruity flavors 10–15 s after spitting

[a] Allen's Chico, Nestlé Australia Ltd.

HPLC Analysis. Organic acids (citric, tartaric, succinic, lactic, and acetic), glucose, fructose, acetaldehyde, and ethanol were determined in must and wine samples using a Shimadzu LC-10AT*VP* Ion Chromatograph system. Diluted samples (1:10, 10 μL) were automatically injected onto an Aminex H7X-8H column (300 × 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 were 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 seen for standards of known concentration.

Standard Winery Analyses. Ethanol contents were quantified in wines prior to bottling using a Wine Alcolyzer (Anton Paar). Titratable acidity was determined using a PHM85 Precision pH meter coupled to an ABU80 autotitrator (Radiometer, Copenhagen).

Wine Sensory Analysis. A preliminary benchtop evaluation was conducted using a panel of seven experienced wine tasters (Provisor Pty. Ltd.) to determine whether detectable differences existed between fermentation replicates. Wines were presented in identical order in coded

Table 2. Key Compositional Parameters of Juice and Wine from Control (C) and Frozen (F) Musts Immediately after Crushing, Prefermentation (Post-Storage), and Prebottling in 20, 50, or 300 kg Aliquots[a]

	after crushing						prefermentation		prebottling		
treatment	weight (kg)	Baumé	pH	total phenolics (g of GAE[b]/L)	total anthocyanins (g/L)	glucose + fructose (g/L)[c]	total phenolics (g of GAE[b]/L)	total anthocyanins (g/L)	ethanol (g/L)	phenolics (g of GAE[b]/L)	anthocyanins (g/L)
C20	19.8	$14.5^{f,g,h}$	3.97	$17.2^{b,f}$	0.28^{b}	297	$21.3^{a,c,d}$	$0.65^{a,f,g}$	$152.6^{d,f,g}$	34.5^{b}	1.46^{b}
F20	19.8	$12.5^{b,e,h,j}$	3.95	$11.8^{d,e,f}$	0.2^{e}	291	33.4^{d}	$1.40^{c,e,g,i}$	$156.6^{c,g,i}$	$35.4^{e,f,h}$	1.47^{e}
C50	49.9	$14.5^{c,d,e,g,i}$	3.96	$15.2^{a,e}$	0.23^{a}	271	24.8^{b}	$0.68^{d,e}$	$156.7^{a,d,e}$	$38.0^{a,f}$	$1.42^{a,f}$
F50	49.9	$12.5^{a,d}$	3.96	15.0^{c}	0.28^{d}	275	$40.4^{b,c}$	$1.87^{b,d,f,h}$	$156.8^{b,f,h}$	$29.9^{d,g,h}$	$1.34^{d,f}$
C300	303.7	$14.3^{a,b}$	4.02	$22.4^{a,b,c,d}$	$0.39^{a,b,c,d,e}$	305	32.8^{a}	$1.02^{a,b,c}$	$154.5^{a,b,c}$	$53.1^{a,b,c,d,e}$	$2.08^{a,b,c,d,e}$
F300	277	$14.3^{c,f,i,j}$	4.06	18.0	0.22^{c}	316	29.1	$0.94^{h,i}$	$154.9^{e,h,i}$	$38.4^{c,g}$	1.46^{c}

[a] Values are the mean of triplicate fermentations. Pairs of values within a given column identified by the same superscripted letters are significantly different (t-test, $p = 0.05$). [b] Gallic acid equivalents. [c] No significant differences found between the values in this column.

ISO standard tasting glasses (30 mL) and assessed at room temperature under white fluorescent lighting. This preliminary analysis confirmed uniformity of the replicates; therefore, individual replicates were used at random. A quantitative descriptive analysis (*16*) of wines followed and was conducted by using an experienced 10-member panel who met once a week for four weeks to develop an attribute list for 13 aroma and 10 by mouth (flavor and mouth feel) characteristics to describe the red wines (**Table 1**). Panellists were trained to recognize these sensory attributes and calibrate their intensity measurement framework in the samples that were presented to them. Each of these terms had an agreed definition and a suitable reference standard that was presented at each sensory session.

In one formal rating session, samples were served at 22−23 °C under orange lights to minimize visual cues. Wines were presented in triplicate in random order across the panelists in three-digit-coded, covered ISO standard tasting glasses (25 mL). The panelists rated each of the attributes on an unstructured 10 cm line scale, with anchors of "low" and "high" placed at 1 and 9 cm, respectively. FIZZ software (Version 2.10c, Biosystemes, Couternon, France) was used for the collection of all sensory data.

GC-MS Determination of Ethyl Acetate. Determination of ethyl acetate content of the wines was achieved using a method derived from that reported by Rodriguez-Bencomo et al. (*17*). 2-Methyl-3-buten-2-ol was used as an internal standard. Chromatography was performed using a Hewlett-Packard 6890 GC equipped with a Hewlett-Packard 5973 MS system and an SGE BP20 column (30 m, 0.32 mm ID × 0.25 μm film thickness).

Statistical Analysis. Data was analyzed using analysis of variance (ANOVA) and was performed using FIZZ and XLStat (Version 7.5.2, Addinsoft). In the case of compositional data, paired t-tests were performed to determine the significance of any differences seen between treatments and were conducted using Prism (Version 4, GraphPad Software).

RESULTS

Prefermentation Consequences of Must Freezing. To establish whether making wine from fresh (control) and frozen-stored musts caused differences in wine quality, grapes treated accordingly were fermented on three scales (20, 50, and 300 kg). The average values for several parameters were measured after crushing, prefermentation (i.e., after must storage), and prebottling (**Table 2**). Although significant differences ($p \leq 0.05$) were observed for some parameters, within and across treatments and scales, generally these were small. Baumé measurements for the 20 and 50 kg lots were approximately 2° Bé lower for the frozen-stored musts compared to those from control musts. Such differences were not however reflected in the glucose and fructose contents of the musts or the ethanol contents of the wines. Total phenolics contents (as GAE) were highest for the 300 kg aliquot of control must (22.4 g/L) and lowest (11.8 g/L) for the frozen 20 kg aliquot. Total anthocyanins averaged around 0.24 g/L for all treatments except the 300 kg aliquot of control must that contained 0.39 g/L. Titratable acidity ranged from 4.2 to 5.6 g/L across the treatments (data not shown).

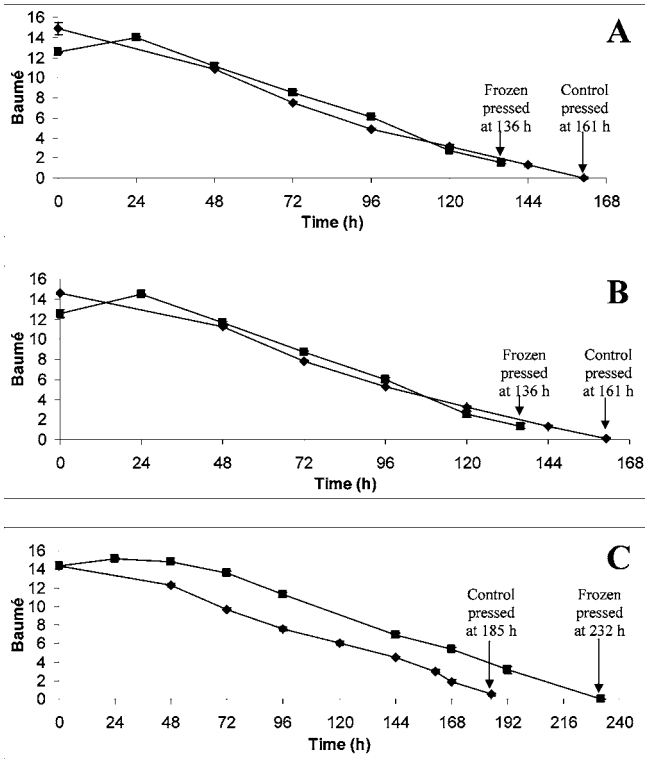

Figure 1. Comparisons of Baumé measurements taken throughout fermentation of control and frozen musts are shown for the 20, 50, and 300 kg scales (panels **A**, **B**, and **C**, respectively). Arrows indicate the point at which time ferments were pressed. Error bars (hidden behind the symbols in most cases) represent the standard deviation of triplicate ferments. N.B. Baumé was not determined beyond 136 h (20 and 50 kg) or 192 h (300 kg) in the frozen must experiments as the wines were ready to press according to standard winery practices. Any delay in pressing was due to accessing processing equipment, which was under heavy demand at that time. (◆) Control must and (■) frozen must.

Fermentation of Control and Frozen Musts. Once warmed to 20 °C, all musts were inoculated and then allowed to ferment under similar conditions. Baumé curves of fermentations from control musts showed that the 20 and 50 kg fermentations progressed at a similar rate (approximately 2.1 °Bé/day) and approached dryness after 161 h (**Figure 1A**,**B**). Similar rates

Table 3. Glycerol, Acetaldehyde, and Organic Acid Content of Control (C) and Frozen (F) Musts and the Resulting Wines[a]

		concentration (g/L)					
treatment		glycerol	acetic acid	citric acid	lactic acid	succinic acid	tartaric acid
C20	crush	0.02	0.31	0.30	0.79	5.82	2.29
	preferm	0.04	0.13	0.70	1.12^{f}	6.73	2.84^{j}
	postferm	7.6^{h,i,j}	0.17	1.28	3.10^{l}	8.66	2.61^{l}
C50	crush	0.08	0.03	0.56	0.97^{d,e}	5.89	1.90
	preferm	0.17	0.09	0.90	1.55^{j}	6.64	2.35^{g,h,i}
	postferm	11.34^{a,e,f,g}	0.16	2.36	4.51	11.12	2.31^{k}
C300	crush	0.03	0.16	0.59^{a}	1.03^{a,b,c}	6.02	1.79^{a,b,c}
	preferm	0.12	0.18	1.29^{b,c,d}	2.01^{f,g,h,i}	7.42	2.22^{d,e,f}
	postferm	10.15^{a,b,c,d}	0.09	7.73	6.43^{k}	12.76	3.74^{k,l,m}
F20	crush	ND	0.24	0.30	0.69^{c}	6.34	1.16^{c}
	preferm	ND	0.30	0.31^{d}	0.85^{i}	6.84	1.44^{f,i}
	postferm	8.11^{d,g,j}	0.59	1.80	4.31^{l,n,o}	8.00	3.84
F50	crush	ND	ND	0.23^{a}	0.49^{b,e}	5.64	1.04^{b}
	preferm	ND	ND	0.25^{c}	0.67^{h,j}	5.85	1.28^{e,h,j}
	postferm	8.24^{c,f,i}	ND	1.61	3.46^{m,o}	7.89	3.28
F300	crush	ND	ND	0.24	0.45^{a,d}	5.39	1.15^{a}
	preferm	ND	ND	0.28^{b}	0.80^{g}	6.52	1.65^{d,g}
	postferm	6.43^{b,e,h}	ND	1.79	2.53^{k,m,n}	7.33	3.08^{m}

[a] Values are the mean of triplicate ferments. Prefermentation determinations of tartaric acid occurred prior to any acid additions. Pairs of values within a given column and for the same time point identified by the same superscripted letters are significantly different (t-test, $p = 0.05$). ND, not detected by HPLC.

were calculated for the frozen-stored 20 and 50 kg (approximately 2.3 °Bé/day) (**Figure 1A**,**B**). Both the 300 kg control and the frozen musts fermented much more slowly (approximately 1.8 and 1.5 °Bé/day, respectively), with the 300 kg control wine reaching dryness at about 185 h (**Figure 1C**). In contrast, the duration of the 300 kg frozen-stored must fermentations required some 50 h longer (**Figure 1C**). None of the frozen-stored must fermentations were completely dry before being pressed, and ethanol concentrations at the time of pressing had reached about 100 g L^{-1} for the 20 and 50 kg batches, and about 120 g L^{-1} for the 300 kg batches. However, at the conclusion of the ethanolic and malolactic fermentations, analysis of the six wines revealed them to be dry (<2 g/L sugar by HPLC, data not shown) and showed little difference in ethanol content (i.e., 152.6−156.8 g/L; **Table 2**).

HPLC Analyses. Glycerol, acetaldehyde, and various organic acids were measured in all samples immediately after crushing, prefermentation (post-storage in case of frozen-stored must), and prebottling (**Table 3**). Small amounts of glycerol were found in all control musts, with increased amounts in all control samples post-fermentation. No glycerol was detected in the crushed and preinoculation samples of the frozen must. Acetaldehyde contents of all samples were low and with no clear trend (data not shown). No acetaldehyde was detected post-fermentation in any wine where it had been detected in earlier samples. Acetic acid was present in all control musts and wines as well as all 20 kg frozen samples. Citric, lactic, and succinic acids increased in all cases in the post-fermentation samples compared to earlier ones. While the final amounts of the organic acids differed across the treatments, values were of the same order of magnitude and showed no consistent trends for the grape treatments or fermentation volumes.

Total Anthocyanin and Phenolic Analysis. In addition to determining total anthocyanins and total phenolics after crushing and prefermentation (**Table 2**), the same measures were made prior to bottling the wines. As expected, both total phenolics and anthocyanins increased significantly throughout the fermentation. Notably, the highest amounts of both anthocyanins (2.1 g/L) and phenolics (53.1 g/L) were seen at the end of the 300 kg fermentation of the control must. However, no consistent trends were seen for a given fermentation volume, but significant differences were found for wines made from control musts and when all scales and treatments were compared.

Descriptive Sensory Analysis. One of the ultimate objectives of experimental winemaking on a small scale is to produce wines that are comparable both sensorily and compositionally to those produced on larger or industrial scales. Thus, to further characterize the wines produced from the treatments investigated, a descriptive sensory analysis was performed. Sensory scores for each of the aroma and palate attributes agreed upon by the panel (**Table 1**) are shown graphically (**Figure 2**). The sensory profiles of the wines were similar, and importantly many undesirable attributes, such as volatile acidity, metallic, and bitterness, were low in all cases. Of the 23 attributes rated in the six wines, highly significant differences ($p = 0.05$) were perceived between the wines in only four of the characteristics (**Figure 2**). In terms of fresh, fruity characteristics, there was no clear differentiation between the wines made from control musts and those made from frozen must, although the control must fermented on the 300 kg scale exhibited a lower intensity of this characteristic. The same was true in both freshness of aroma and red berry aroma characteristics. Tannin hardness and burnt/smoky aroma were more intense in the control must fermented in 300 kg aliquots.

A key difference that might be expected across the fermentation scales utilized relates to the ingress of oxygen into the fermentations. Greater oxygen availability in the smaller fermentations might in turn lead to elevated volatile acidity (*1*). No such eventuality was evident from the determinations of the acetic acid content of the wines (**Table 3**) or from their sensory profiles (**Figure 2**). However, volatility was an attribute noted by the sensory panel. For this reason, measurement of ethyl acetate content was also performed. Concentrations were found to range from 59 to 123 g/L with no consistent trend for fermentation volume or must treatment (data not shown).

DISCUSSION

The notion of using frozen fruit or must for research winemaking investigations outside of vintage, which are expected to be representative of traditional grape processing and winemaking, has received modest attention, particularly in terms of red winemaking (*4*, *6*, *7*). Longer term storage trials of intact

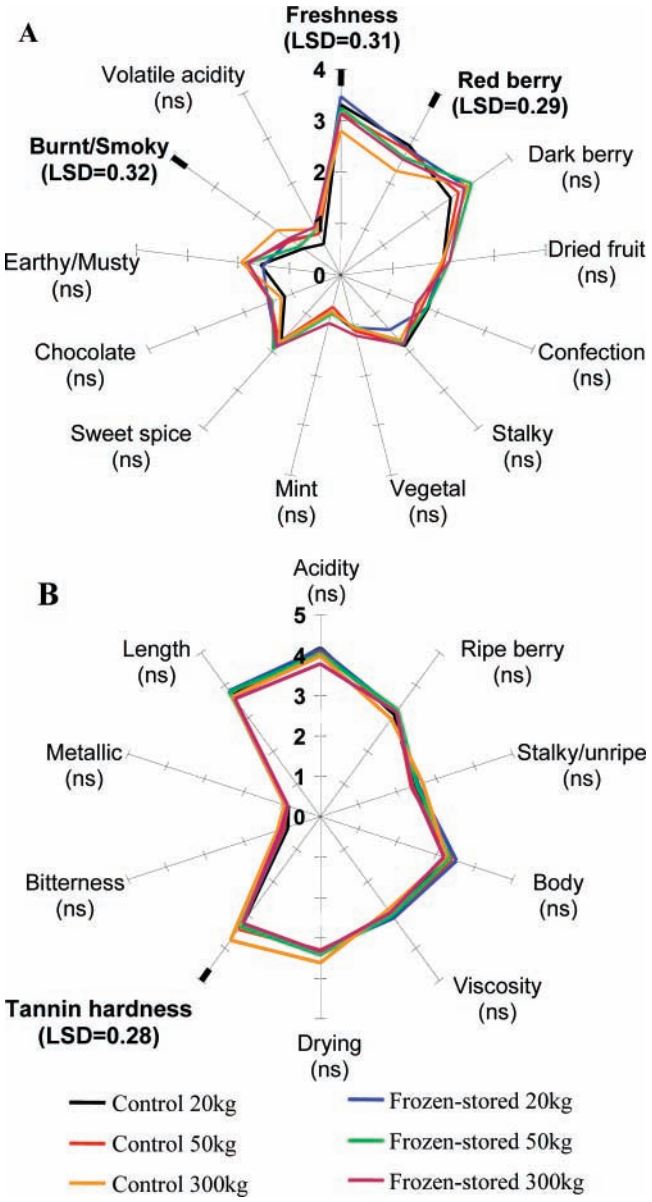

Figure 2. Plots showing the intensity of aroma (**A**) and palate (**B**) attributes of wines made from control and frozen-stored musts at scales of 300, 50, and 20 kg. The data presented are an average of triplicate ratings for each representative wine. See text for full details of sensory analysis; ns, not significant at $p = 0.05$. The size of the LSDs are indicated by the thick bars at the end of the axis with the numerical value also given in the figure.

berries suggest that only between 5 and 10% of total anthocyanins are lost during storage at −18 °C (*8*). In this study, we sought to provide quantitative data on the consequences of the use of frozen compared to fresh must for the production of experimental wines in 20, 50, and 300 kg aliquots. Compositionally, while some statistically significant differences were found, neither the must freezing nor the scale of the fermentation experiment produced substantial or persistent compositional differences at the post-crushing, prefermentation, or prebottling stages. Differences seen in Baumé readings post-crushing were not seen in sugar or final ethanol contents (**Table 2**) suggesting that the differences were not solely due to sugar content.

Monitoring the progress of fermentation revealed a reality of experimental winemaking that it is commonly difficult to achieve consistency across fermentation scales. Although the 20 and 50 kg fermentations (held in a 20 °C room) progressed with similar kinetics, the 300 kg fermentations conducted at ambient temperature (i.e., between 16.3 and 18.4 °C) were slower. While variations in fermentation kinetics are likely to have arisen from such temperature differences, their importance for wine composition as determined in this study (**Tables 2** and **3**) appears minor. In any case, had closer control been available, its application might itself have been problematic. The greater agitation likely to be needed to effect tighter temperature control of the 300 kg fermentations may well have resulted in greater maceration and thereby altered extraction of the skins and hence composition of the final wine (e.g., *18*, *19*). On the other hand, three-dimensional uniformity of temperature might also be undesirable. Our unpublished findings (Block, Schmid, and Jiranek) and the work of others (*20*) confirms the existence of complex temperature gradients in commercial red wine fermentations, which are likely to be difficult to reproduce on a smaller scale. Further work is clearly required in this area both to determine the importance of temperature distribution on wine composition and to develop research systems capable of accurately modeling such profiles.

Additional evidence for the absence of major differences between the treatments investigated in this study came from further chemical analysis of samples taken at various time points across crushing and prebottling, as well as sensory analysis of the resulting wines. There was no suggestion that freezing and subsequent thawing resulted in markedly elevated organic acid contents of the wines. While acetic acid was more readily detectable in the control must (**Table 3**), perhaps suggesting a greater degree of microbial activity in these musts, the delay in performing acetic acid quantitation prevented the timely conduct of any microbial analysis. Prefermentation, glycerol was also detected in all early samples from control musts. Even though it is suspected that the combination of must storage at or below 0 °C with PMS would have largely eliminated growth of indigenous microorganisms, future studies should examine the question of prefermentation microbial load specifically.

Total phenolics and total anthocyanins indicated some differences between treatments, with control musts tending to have higher values compared to the frozen-stored equivalent (**Table 2**). Overall, however, the increase in these parameters during the fermentation agrees with that widely reported to occur in red wine fermentations (*1*, *2*). Sensory analysis of the wines revealed broad similarity in their aroma and palate profiles (**Figure 2**) while highlighting some key areas of divergence. Thus, the 300 kg fermentations of control musts were less intensely fresh, with less red berry characteristics and more tannic appearance. All remaining attributes were similar across the treatments. Suggestions by some panel members that volatile acidity was evident in some wines were not supported by determinations of acetic acid or ethyl acetate contents of the wines. Quoted thresholds for ethyl acetate in red wines range from 70 mg/L (*21*) to 160 mg/L (*22*) and more recently up to 198 mg/L (*23*). Ethyl acetate contents determined in this study (i.e., 59−123 mg/L) are below these most recent threshold determinations. Similarly, the threshold for acetic acid (ca. 0.7 g/L; *20*, *22*) exceeds the values typically reported here (undetectable − 0.6 g/L) thereby agreeing with the low score for volatility from the sensory panel.

The findings reported here strongly suggest that despite some statistically significant compositional and fermentation differences between the six wines made from fresh and frozen must at three different scales, they were nevertheless similar with regard to chemical composition and particularly their sensory properties. This fact supports the validity of the freezing of grape

must as a means of preserving fruit for use in winemaking trials outside of the vintage period. In addition, the likelihood that it is possible to achieve winemaking outcomes that are comparable to each other on a 20 and 50 kg scale as well as on the 300 kg scale has also been demonstrated. Of course, further work will be required to confirm the relevance of these findings to other grape varieties and also to define the maximum period of storage of frozen musts.

ABBREVIATIONS USED

PMS, potassium metabisulfite; VA, volatile acidity; TA, titratable acidity; GAE, gallic acid equivalents; LSA, least squares analysis; SO_2, sulfur dioxide.

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