Red wine composition and sensory analysis with different inoculation times for malolactic fermentation

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Abstract

This study was designed to investigate differences in wine sensory and chemical composition arising from altering inoculation times for malolactic fermentation (MLF) in red wine. Wine was made using a blend of Shiraz, Cabernet Sauvignon and Mourvédre grapes inoculated with Saccharomyces cerevisiae EC1118. Oenococcus oeni (Viniflora™ Oenos) starter culture was inoculated either concurrently, at 3.2 Baumé, or when alcoholic fermentation had been completed. There was no change in the rate of alcoholic fermentation across treatments, however, malic acid degradation was moderately slower when inoculation occurred at 3.2Bé. Acetic acid levels in stabilised wine varied between 0.29 grams per litre (dry inoculation) and 0.41g/L (3.2Bé inoculations) but remained below acceptable sensory thresholds. Total anthocyanins and related wine colour measures were improved in wines in which co-inoculated yeast and MLF starter cultures were used, indicating a greater ageing potential for these wines. Sensorial analysis with a trained panel could not distinguish differences between treatments. This research illustrates the potential to vary timing of MLF inoculation for red wine without detrimental effects to finished wine quality.

Introduction

MLF is an important bacterial transformation of L-malic acid to L-lactic acid and may be encouraged by winemaking

practices to occur in red and some white wines through growth of certain lactic acid bacteria (LAB). The benefits of MLF in winemaking are improved microbial stability, reduced acidity, flavour, and textural modification. Desirable flavour changes that may arise from MLF are described as buttery, estery, nutty, sweaty, lactic and oaky [1-3]. Wines that have undergone MLF are also considered to have greater length of flavour, more body and enhanced sensory appeal [4]. Evidently, the sensorial benefits of MLF in wine vary considerably according to the strain of O. oeni, grape variety and the winemaking processes used during production. A number of starter cultures of O. oeni are commercially available and these strains are typically added to the wine following completion of the alcoholic fermentation by Saccharomyces cerevisiae [5]. However, even with the use of commercial starter cultures, delayed or stuck MLF can arise from poor or inappropriate nutrient management, the production of inhibitory compounds from yeast, or the extremely harsh physicochemical properties of the wine matrix. Timely completion of MLF enables more efficient winemaking practices and blending decisions to take place.

Problems may arise from late inoculation of starter cultures into wine for MLF. An unexpected temperature drop combined with nutrient depletion and high ethanol concentration can make the management of MLF challenging. It is not uncommon for wineries to have some wines that have not completed MLF prior to winter, and these must be left with low concentrations of sulfur dioxide until seasonal temperatures rise, which allow bacterial growth and metabolism to resume, or have expensive temperature control to ensure appropriate conditions conducive for growth of the MLF organisms. The cellaring of wines without adequate sulfur dioxide to prevent oxidation and limit growth of micro-organisms can be deleterious to wine composition, as spoilage organisms can commence growth along with O. oeni when warmer temperatures permit [6].

On the other hand, the growth of certain LAB, including O. oeni, early in the winemaking process is considered by some to be undesirable for wine composition. Increased acetic acid and D-lactic acid levels may arise from heterofermentative degradation of fermentable carbohydrates [7], hence the reluctance by some winemakers to use MLF starter cultures early in the winemaking process. Inhibition of bacterial growth by nutrient depletion arising from yeast metabolism may be augmented by the production of inhibitory excretory products of yeast growth, the most obvious being ethanol, sulfur dioxide, low molecular proteins and medium chain fatty acids [8, 9].

Several reports have recently described the microbial interactions of concurrent growth of S. cerevisiae and O. oeni in either model wines – wine made from white grape varieties or bench top vinifications [6, 10]. Screening methods to assess the potential interactions of the growth of S. cerevisiae and O. oeni are also well described in chemically defined media [11]. Limited or no sensorial investigations have been published that describe the impact of concurrent inoculation of S. cerevisiae and O. oeni starter cultures and the subsequent growth of the starter cultures in red wines under winemaking conditions. In this paper, we describe key compositional attributes to red wine when starter cultures of S. cerevisiae and O. oeni are used concurrently, and compare these wines to those inoculated for MLF near the end and following completion of the alcoholic fermentation. Comprehensive sensorial assessment of the wines is also reported.

Materials and method

Grapes

Approximately 520 kilograms of grapes were harvested from the Riverina, New

South Wales, destemmed, crushed and stored at -18°C until fermentations were commenced. Grape varieties and volumes of the final composition of the blend were Shiraz (83%), Cabernet Sauvignon (8.5%) and Mourvédre (8.5%). Grapes were equally distributed to nine variable capacity tanks of 100L capacity and samples taken for measurement of pH and titratable acidity (TA).

Fermentation and starter culture inoculations

All winemaking equipment was kept separate for each treatment and sanitised immediately prior to and after use to eliminate indigenous winery organisms and avoid cross-contamination. Treatments were: (1) co-inoculation of yeast and bacterial starter cultures for MLF (CI); (2) inoculation for MLF in the last third of alcoholic fermentation (AI); (3) inoculation for MLF following completion of alcoholic fermentation, determined when residual sugar levels were less than 0.5g/L and wine was pressed off skins (DI). Three replicates were performed for each treatment.

S. cerevisiae (EC1118) was used to inoculate each tank. MLF starter culture (Vinflora™ Oenos) was prepared by resuspending a fresh sachet of organisms in a 250-millitre aliquot of a ferment corresponding to the treatment to be inoculated, mixing and adding 50mL of this suspension to each treatment replicate corresponding to the manufacturer's recommendations for inoculum density.

Ferments were plunged; specific density and temperatures determined using an Anton Paar DMA 35n twice daily. Must samples were taken every 48 hours to measure malic acid concentration. Seven days post yeast inoculation, all wines were pressed off skins. At the end of MLF, wines were racked and a 50-parts-per-million sulfur dioxide addition made. All wines were cold stabilised and tested for protein stability. Wine was racked a second time and free sulfur dioxide levels adjusted to a minimum of 30ppm prior to packaging into 750mL bottles, snow dropped with a small pellet of dry ice and sealed using ROTE closures.

Laboratory analysis

Glucose, fructose, acetic and malic acids were measured by enzymatic procedures in miniaturised format. Ethanol concentration was determined in duplicate for wines using an Anton Paar Alcolyzer. Spectral measures at 280 nanometres, 420nm and 520nm were taken on finished wine in 1-militre or 10mm matched quartz cuvettes against water blanks and following treatment with acetaldehyde, sulfur dioxide or hydrochloric acid solutions. A Shimadzu UV-2101PC spectrophotometer was used for measuring absorbance and all values were corrected to a 10mm path length.

Sensory analysis

A sensory panel comprising of four females and eight males, 25 to 43 years old, were trained over four weeks. All treatment replicates were used during training and testing. Following training, the sensory panel completed descriptive analysis on the test wines, generating aroma standards appropriate for the wines (Table 1). The formal evaluation of the wines was undertaken over three sessions. Each wine representing the three treatment levels and three replicates for each treatment was assigned a three-digit random number by Compusense™ 5.0, which was transcribed onto ISO XL-5 glasses. Each panelist was presented with three wines at each session in a random order but were presented with all wines over the course of the evaluation. Panelists were instructed to rate the wines in terms of blackberry, cherry, stalky, acidity, aroma and flavour intensity using a 1 (low) to 6 (high) preference scale. Overall quality was ranked using a 1 to 6 preference scale. All evaluations were conducted under white fluorescent lights at ambient temperature (approximately 22°C) in individual tasting booths.

Statistical analysis of sensory data

A mixed model ANOVA was performed to determine sensory panel reliability by comparing repeated judgements for each attribute. To ensure the panel was discriminating between the attributes, ANOVA was used to compare attribute means. Having determined that the sensory panel performed acceptably (reliably and capable of discrimination) to determine the effects of varying the timing of inoculation for MLF, ANOVA was then applied to test the main effects for treatment (CI, AI and DI) and wine replicates (three separate wine replicates). To establish the degree of similarity between the wines, Pearson Product-Moment correlations were determined.

Results

Chemical analysis

Chemical analysis of the wines is shown in Table 2. Homogenised grape samples yielded a commencing Bé of 14.9, pH 3.64 and TA of 6g/L. Inoculation of the fermentations with

Table 1. Aroma standards for panelist training

Aroma	Stock	Preparation of standard from stock
Cherry	Admiral canned raspberry brine Admiral canned dark cherry brine	500µL added to 50mL red wine base 250µL added to 50mL red wine base
Blackberry	Monbulk blackberry jam	50g jam added to 50mL red wine base
Stalky	Geranium stalks 3.0g, Cut grass 0.5g soaked in 20mL ethanol	4mL added to 50mL red wine base

Table 2. Analytical results of finished wines. Values are mean ± SE. Significant differences between treatments are indicated across rows by letters.

	MLF inoculation concurrent with yeast (treatment CI)	MLF inoculation during alcoholic fermentation (treatment AI)	MLF inoculation post alcoholic fermentation (treatment DI)
Ethanol %v/v	13.76 ± 0.04	13.69 ± 0.06	13.78 ± 0.08
pH - initial	3.64 ± .01	3.64 ± 0.01	3.63 ± 0.01
pH - final	3.86 ± 0.03	3.84 ± 0.02	3.83 ± 0.01
Titratable Acidity - initial§ g/L	5.95 ± 0.01	6.01 ± 0.03	6.01 ± 0.03
Titratable Acidity - final g/L§¶	5.61 ± 0.05	6.02 ± 0.12	5.89 ± 0.06
Free SO_2 ppm	31 ± 0.6	36 ± 1.9	31 ± 0.7
Total SO_2 ppm	82 ± 1.5	87 ± 2.9	83 ± 0.3
Ratio Free SO_2:Total SO_2	0.38 ± 0.01	0.41 ± 0.02	0.38 ± 0.02
Acetic Acid g/L¶	0.40 ± 0.01	0.41 ± 0.01	0.29 ± 0.01
Wine Colour Density	5.32 ± 0.08	5.58 ± 0.68	5.39 ± 0.06
Wine Colour Hue†	0.65 ± 0.01	0.64 ± 0.06	0.70 ± 0.01
Degree Red Pigmentation %	10.6 ±0.2	13.4 ± 2.0	12.5 ± 0.3
Total Red Pigments au¶	30.33 ± 0.33	25.67 ± 0.33	25.3 ± 0.88
Total Phenolics au	57.67 ± 1.20	57.67 ± 6.17	51.00 ± 1.16
Total Anthocyanins mg/L¶	564 ± 4	465 ± 8	453 ± 18
Ionised (coloured) Anthocyanins mg/L†	37.3 ± 1.2	39.7 ± 11.7	31.0 ± 1.2
Ionised (coloured) Anthocyanins %	6.6 ± 0.2	8.4 ± 2.3	6.8 ±0.1
Decolourisation of Anthocyanins au	2.37 ± 0.09	1.87 ± 0.53	2.27 ± 0.03
%Total Anthocyanins in ionised (coloured) form corrected for SO_2¶	14.97 ± 0.09	16.50 ± 0.20	16.83 ± 0.39
Chemical Age¶	0.045 ± 0.000	0.057 ± 0.001	0.064 ± 0.002

MLF starter culture during the alcoholic fermentation was performed when the Bé was 3.2, corresponding to approximately 20% of remaining fermentable sugars. Bé and fermentation temperatures during the alcoholic fermentation are illustrated in Figure 1A, and the duration of malic acid degradation and fermentation temperature for each treatment in Figure 1B. No differences between treatments in the rate and duration of alcoholic fermentations were apparent, indicating no inhibition of yeast activity by O. oeni. MLF is shorter in wines inoculated concurrently with yeast (CI) or at the completion of alcoholic fermentation (DI) compared to wines inoculated at a Bé of 3.2 (AI).

Acetic acid is slightly higher in treatments in which concomitant growth of S. cerevisiae and O. oeni occurred, i.e. treatments CI and AI. While the acetic acid levels were raised in these two treatments compared to the DI wines, the concentration is not above that considered objectionable [12] or exceeding legal limits in red wine in Australia.

The concentrations of anthocyanins were significantly increased in co-inoculated wines (treatment CI). Associated with higher levels of anthocyanins in these wines was improved total red pigments, decreased percentage of red pigmentation and decreased ionised anthocyanins corrected

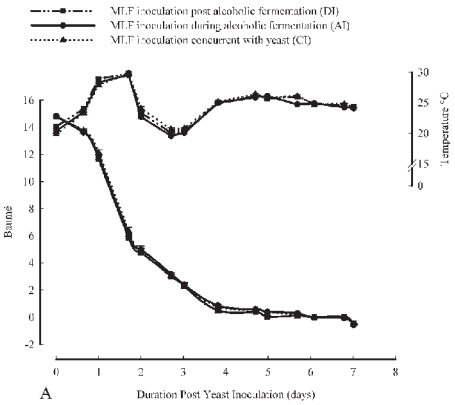

Figure 1A. Alcoholic fermentation and temperature profiles of ferments with differing inoculation times for malolactic fermentation.

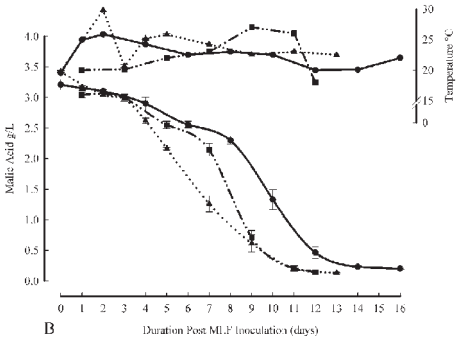

Figure 1B. Malolactic fermentation (MLF) and temperature profiles with different inoculation times for MLF.

for sulfur dioxide, compared to wine which was inoculated for MLF during (treatment AI) and after alcoholic fermentation had ceased and wine pressed off grape skins (treatment DI). Other wine analysis did not vary across treatments.

Sensory analysis

Mean preference scores of the sensorial attributes tested by panel members for the wines of each treatment are shown in Figure 2. Sensory panel performance was considered reliable and consistent across all sensorial attributes from the results of the mixed model ANOVA (Table 3). ANOVA test for discrimination of the attributes by panelists also indicated that all members were consistently able to discern the sensory attributes. ANOVA of the attribute means revealed that the panel rated attributes differently.

ANOVA testing the main effect for treatment was not significant, showing that there was no statistical difference between the three treatments investigated in the current experiment. The main effect for wine replicates was also not significant, showing that there was no statistical

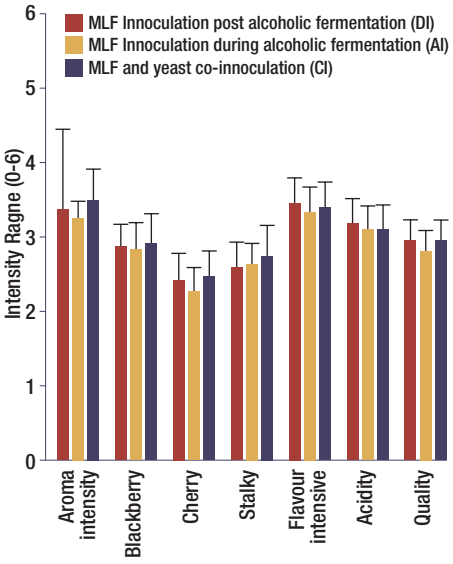

Figure 2. Hedonic scores of sensorial attributes ranked for each wine by panel members. Attributes are shown with means ± standard errors.

difference between the three wine replicates. The treatment by wine replicate interaction effect was also not significant, enabling the main effects for treatment and wine replicate already reported to be interpreted without ambiguity; there were no statistical differences between treatments for any of the attributes tested and that this finding held for all wine replicates made.

Table 3. Calculated F values of the mixed model ANOVA used to determine reliability and consistency of the sensory panel performance. Calculated F statistics from the analysis are all non-significant at α = 0.001, demonstrating reliable judgements across all sensorial attributes.

Sensorial Attribute	Replicate* Sample vs Error F values	Significance
Aroma Intensity	1.377	p<0.001
Blackberry	1.387	p<0.001
Cherry	0.770	p<0.001
Stalky	2.187	p<0.001
Flavour Intensity	1.070	p<0.001
Acidity	2.557	p<0.001
Overall Quality	1.620	p<0.001

The degree of similarity between the wines was positively correlated and all correlations were significant at α = 0.01; therefore treatments were statistically similar, suggesting that each treatment was rated the same way by the sensory panel.

Discussion

The scope of this investigation was to determine the feasibility of conducting simultaneous alcoholic and malolactic fermentations using a combination of starter cultures commonly employed for red wine production. Little variation was apparent for the duration of alcoholic fermentation for each treatment (Figure 1A), indicating that there was no considerable inhibition on yeast metabolism by early inoculation of MLF starter culture of O. oeni. This observation supports other researchers who did not observe any changes to yeast activity in the presence of MLF starter cultures [13]. The time for completion of MLF is, however, different according to the treatment. O. oeni starter culture added to the wine either concurrently with the yeast inoculation, or when the alcoholic fermentation was finished (treatments CI and DI), had completed MLF by Day 12, whereas MLF inoculation during alcoholic fermentation (treatment AI) required an additional four days. Differences in the rate of malic acid decomposition were also evident between treatments, with the fastest rates for malic acid transformation corresponding and slightly lagging to temperature spikes for each treatment (Figure 1B). Temperature is likely to have been a significant contributor to the change in rate of malic acid decomposition. The slight lag in the flux of malic acid as wine temperature approaches the 25°C optimum for growth O. oeni [14] suggests that accumulation of bacterial biomass, and therefore intracellular malolactic enzyme, is necessary for MLF.

Higher wine temperatures will also increase the efficiency of the malolactic enzyme [5].

Loss of bacterial viability when MLF cultures are added during alcoholic fermentations has been reported, although successful MLF is still possible [15]. Yeast metabolic activity will be highest during fermentation, and it can be reasonably expected that production of inhibitory compounds such as ethanol and sulfur dioxide will be greatest at this time [16]. Ethanol has an inhibitory impact upon the growth of O. oeni at concentrations greater than 6% v/v, although the effect is strain dependant [5], hence an advantage of early inoculation with MLF starter cultures is the gradual acclimatisation of the bacteria to rising alcohol levels [17], along with greater levels of organic acids, which improve ethanol tolerance [18]. Medium chain fatty acids derived from yeast autolysis are generally higher in concentration if yeast lees are allowed to accumulate [19]. Therefore, expeditious inoculation for MLF following completion of alcoholic fermentation is also warranted if winemaking practices prevent early inoculation.

Of considerable interest is the moderately higher level of total phenolics and greater levels of anthocyanins in wines with early addition of MLF cultures. The proportion of ionised anthocyanins was moderately lower in co-inoculated wines after correction for sulfur dioxide. These results indicate that extended bacterial contact with grape skins may increase anthocyanin extraction. Colour measures were also improved in co-inoculated wines, potentially indicating a greater ageing potential.

Anthocyanins are present in the grape skin cell walls and vacuoles. Recent efforts to determine if strains of O. oeni have the required enzymes for improving anthocyanin release from grape skins were largely unsuccessful [20], suggesting that other mechanisms may exists in which O. oeni can enhance red wine colour.

Sensory panelists performed reliably and with discrimination. Individual panelists consistently rated the sensory descriptors of each wine at similar scale points across all sensory assessments, and were also able to rate without ambiguity each sensory descriptor in all sittings. Despite this, no significant sensory differences are apparent between the three treatments for any of the attributes. Chemical analysis of the wines showed a difference in the levels of acetic acid found in the treatments (refer to Table 2). All levels were well below the reported sensory threshold of 0.74g/L [21], with the highest being 0.41g/L. Results of the sensorial assessment support the assertion that the levels of acetic acid were below sensory threshold, and in these wines have little consequence to the finished wine composition. Correlation was used to show that the sensorial perception of each treatment was statistically similar. These findings provide strong argument that the time of MLF inoculation did not significantly influence the final wines. Given that a trained panel could not detect differences, it can be assumed that untrained assessors (e.g. wine consumers) would also fail to detect any differences between the three inoculation treatments. Therefore, production of red wine styles in which MLF is desirable for stabilisation or acid reduction could be modified when using the combination of starter cultures in this experiment, so that early or concurrent inoculations are made.

The simultaneous inoculation of yeast and bacterial starter cultures for winemaking may not be a universally acceptable or a practical approach in the production of all wine styles. Early completion of microbial wine fermentations is particularly advantageous in wineries located in cooler climates, in those wineries with limited facilities for temperature control of fermentation, and when the demands of winemaking require early blending decisions to be made, such as in the preparation of sparkling wine bases.

An important wine production consideration when inoculating for MLF, regardless of the timing of inoculation, is the suitability and compatibility of yeast and bacterial strains. The use of S. cerevisiae EC1118 and Vinflora™ Oenos, (CHR Hansen) O. oeni, two commonly used starter cultures in the wine industry, appear to be compatible for co-inoculations into red wine. Further work is required to determine how colour, flavour and textural attributes of red wine may be modified by altering inoculation timing of MLF starter cultures.

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