Influence of MLF on the fruity characters of red wine

Bringing chemistry and sensory science together

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This article outlines the results of a study to determine the importance of* Oenococcus oeni *strain selection and wine conditions under which malolactic fermentation (MLF) is conducted to enhance the fruity and berry characters of red wine.

A wine's colour is important, but it is the aroma and flavour that leaves the greatest impression with the drinker. Compounds that contribute to the sensory experience of wine originate from the grape and microbial metabolism during winemaking. Yeasts play a major role in the aroma and flavour of wine, however, bacteria not only are responsible for malolactic fermentation (MLF), but are also contributors to the final sensory experience of wine (Swiegers *et al.* 2005). In addition to reducing wine acidity, MLF also provides microbial stability and offers the opportunity to modify sensory properties of the wine. During MLF, *Oenococcus oeni* metabolism can modify a vast array of wine chemical components, and this can translate into changes in the appearance, aroma and palate of the wine (Swiegers *et al*. 2005). As in the case of the effects of yeast strain on wine composition, many of these alterations arising from MLF are strain-dependent.

The berry fruit aroma characteristics of red wine can vary according to grape variety and winemaking style, and may encompass red berry (e.g. raspberry, strawberry, red cherry) and dark berry (e.g., mulberry, blackberry, plum) attributes (Iland *et al*. 2009). While optimisation of the intensity and balance of such fruity characters remains a highly important factor in the production and overall quality of red wines, the role of MLF in influencing these attributes is not fully understood. Our aim in these studies was to increase the understanding of the role of *O. oeni* strains to influence the berry fruit sensory characters of Cabernet Sauvignon wines through changes in the volatile fermentation-derived compound composition and sensory profile.

EFFECT OF MLF ON 'FRUITY' ESTERS

Wine chemical composition plays an important role in the growth and metabolism of *O. oeni* during MLF. Recent studies have highlighted the ability of *O. oeni* to influence various groups of volatile fermentation-derived compounds (esters, acetates, acids, higher alcohols). Ethyl esters can impart various fruity characters (berry, pineapple and banana) to wine (Siebert *et al*. 2005). Several of our studies in Chardonnay - Shiraz and Cabernet Sauvignon wines have shown that ethyl esters may increase, and there is a general decrease of the acetate esters following MLF (Bartowsky *et al*. 2008, Abrahamse and Bartowsky 2011). The variability of *O. oeni* strains to influence wine ester concentrations following MLF in 2008 Adelaide Hills Cabernet Sauvignon wine is shown in Figure 1. As expected, a strain-dependent increase in ethyl acetate concentrations occurred. However, several esters increase or decrease dependent upon *O. oeni* strain (e.g., 2-methylpropyl acetate, ethyl butanoate and ethyl propanate). Differences in *O. oeni* metabolism will be a reflection of expression variations within the *O. oeni* genome.

Recent work has suggested that red wine berry fruit aroma is a complex interaction between fruity esters, norisoprenoids, dimethyl sulfide, ethanol and other components (Escudero *et al*. 2007). Pineau and colleagues (2009) further identified certain groups of esters that specifically contributed to red berry and blackberry aroma. Studies in Australian Cabernet Sauvignon were undertaken to determine *O. oeni* strain variation in synthesis of esters that contribute to fruity berry aromas, as well as the importance of pre-MLF wine composition and viticultural region (source of Cabernet Sauvignon). Certain esters proposed by Escudero and colleagues (2007) and Pineau and colleagues (2009) were determined and collectively used as a chemical parameter to gauge the potential berry-fruit sensory characters of the wines.

EFFECT OF WINE PH ON MLF

To study the role that *O. oeni* strains play in enhancing the

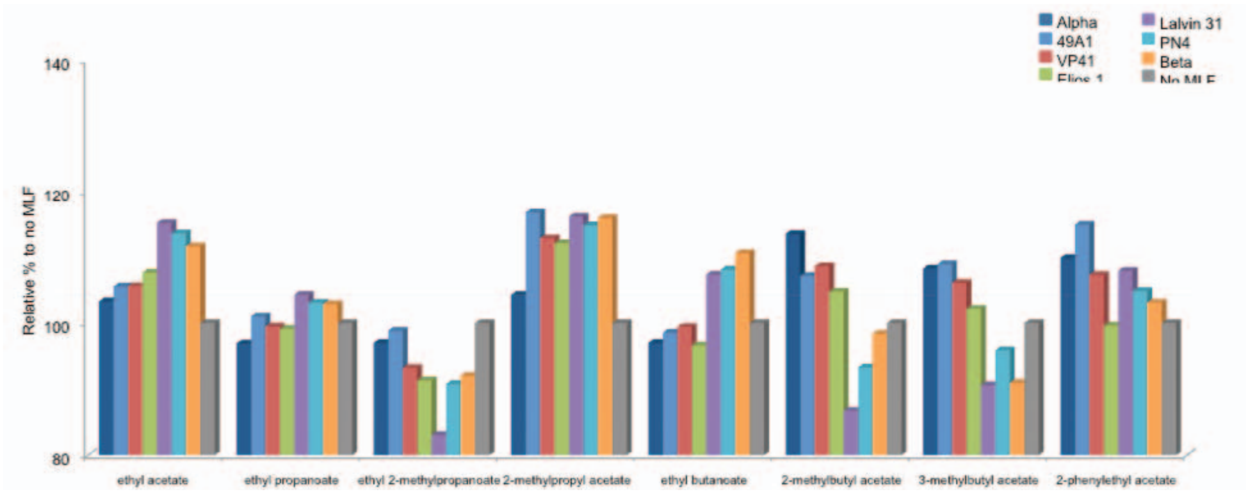

Figure 1. Relative changes in various fruity esters by selected *Oenococcus oeni* strains (seven strains) to no MLF (100%, grey, [right]). MLF was performed in Cabernet Sauvignon (2008 vintage from the Adelaide Hills).

fruity characters in red wine, we investigated the influence of wine pH on *O. oeni* ability to produce volatile fermentation-derived compounds (esters) during MLF. Wine pH is one of the three essential wine chemical parameters (pH, alcohol and SO_2) that will greatly influence the growth and subsequent MLF rate of *O. oeni* (Henick-Kling 1993). To examine the effect of wine pH on the kinetics of MLF, chemical composition and sensory attributes, Cabernet Sauvignon wine was divided into two lots: one lot was adjusted to pH3.3 and the second to pH3.7. These wines were inoculated, in triplicate, with three *O. oeni* strains. As would be predicted, wines at pH3.7 supported the growth of *O. oeni* and a rapid degradation of malic acid, which completed within three weeks, whereas the wines at pH3.3 took approximately 12 weeks to complete MLF. The *O. oeni* population viability was closely linked to the rate of MLF.

The ester profiles of these eight wines were determined and changes relative to wines that did not go through MLF are shown in Figure 2.

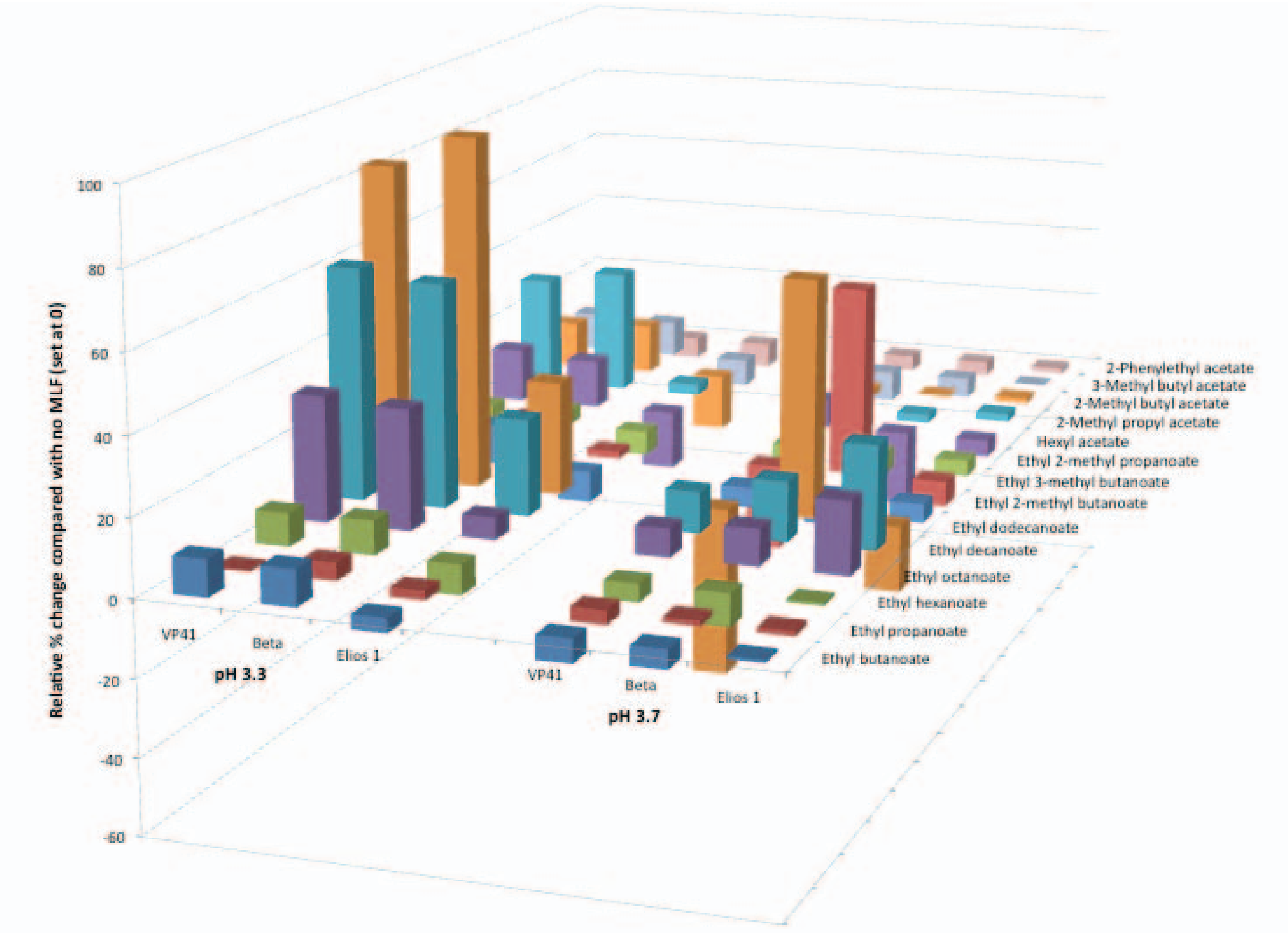

Figure 2. Ester profiles of Cabernet Sauvignon wines (2006 vintage from Clare Valley; pre-MLF wine pH3.3 or pH3.7) following MLF by three *Oenococcus oeni* strains. Changes in concentration are shown as relative percent change to non-MLF (set at 0).

Pre-MLF wine pH was found to have a significant impact on the resultant ester profiles; the lower pre-MLF wine pH (3.3) tended to have a greater increase in these volatiles, in particular the ethyl esters (C4-C8). *O. oeni* strains varied in their abilities to affect the concentrations of the different esters, with *O. oeni* strain Elios 1 exhibiting the least effect in this Cabernet Sauvignon wine, irrespective of pre-MLF wine pH.

LINKING VOLATILE FERMENTATION-DERIVED COMPOUNDS WITH SENSORY ATTRIBUTES – BERRY FRUITY CHARACTERS

The metabolism of *O. oeni* strains at different wine pH values clearly influences their ability to modulate those esters that contribute to berry-fruity aromas in Cabernet Sauvignon wines (2006 wines, Figure 3, see page 31).

Compared with non-MLF wines, the relative total 'berry fruit' ester concentration was much higher for *O. oeni* strains VP41 and Beta at pre-MLF pH3.3, yet, such an increase did not occur at pre-MLF wine pH3.7. Formal sensory descriptive analysis of these eight Cabernet Sauvignon wines also revealed that pre-MLF wine pH was an important factor in influencing fruity-related descriptors. Compared with pre-MLF pH3.7, wines that went through MLF at pH3.3 with *O. oeni* strains VP41 and Beta were rated higher in certain fruity characters, including raspberry aroma (2006 wines, Figure 3). This clearly demonstrates a link between increases in specific berry fruit-related esters and a consequent increase in sensory perception of berry fruit aroma following MLF with specific *O. oeni* strains in these Cabernet Sauvignon wines.

OENOCOCCUS OENI STRAIN PERFORMANCE IN CABERNET SAUVIGNON WINE FROM ONE VINEYARD OVER SEVERAL VINTAGES AND VINEYARDS FROM DIFFERENT VITICULTURAL REGIONS

We were interested in investigating whether *O. oeni* strains behave similarly during MLF in Cabernet Sauvignon wine: (i) from fruit sourced from the same vineyard over several vintages, and (ii) from fruit sourced from different viticultural regions.

Cabernet Sauvignon from one vineyard over several vintages

To investigate vintage variations on MLF, we used Cabernet Sauvignon grapes from the same vineyard in the Clare Valley, South Australia, over two additional vintages (2008 and 2009; alcohol 13.9% and 14.7% v/v, respectively; pre-MLF pH adjusted to

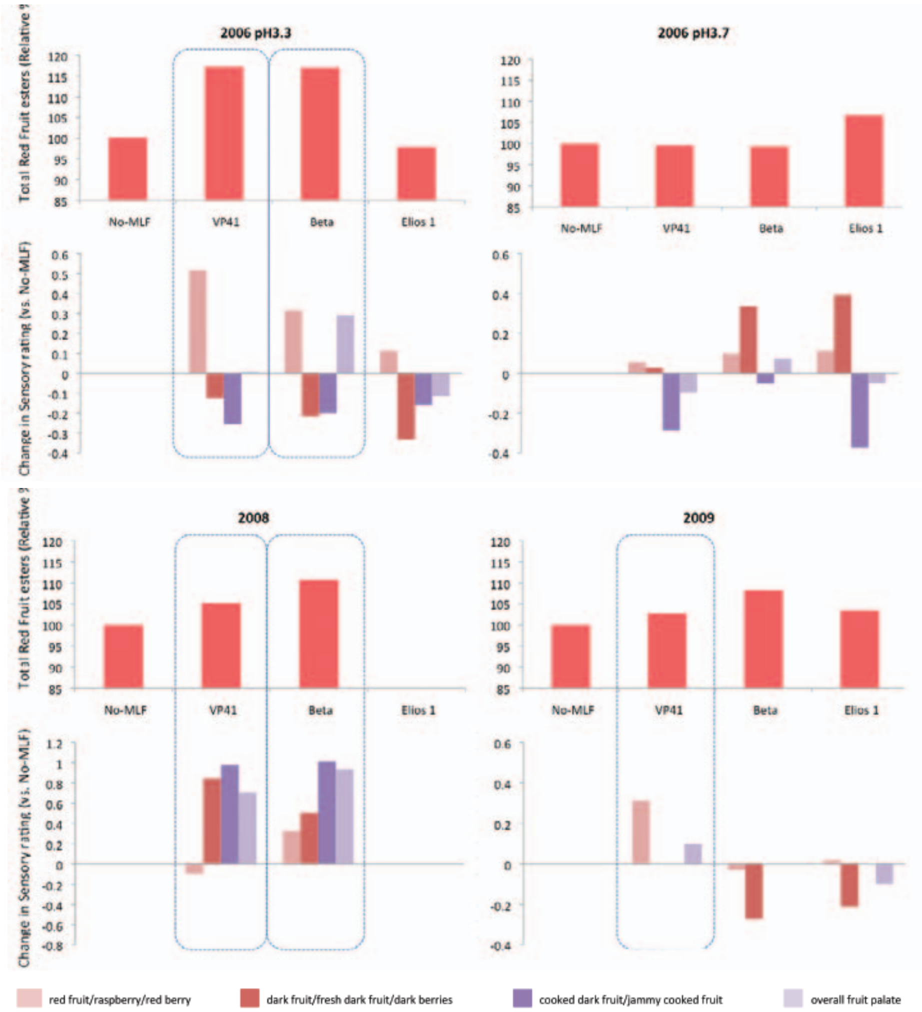

Figure 3. Total berry fruit esters and sensory attribute rating scores expressed as relative percentage change from no MLF, for Cabernet Sauvignon wines following MLF induced with three *Oenococcus oeni* strains. Fruit was sourced from the same vineyard over the three vintages (Clare Valley, South Australia). Pre-MLF wine pH: 2006 – pH3.3 or pH3.7; 2008 and 2009 – pH3.45.

pH3.45). MLF was induced with three *O. oeni* strains and completed within 25 days (2008) and 25-45 days (2009).

Over the three vintages, *O. oeni* strains VP41 and Beta tended to increase consistently the relative concentrations of berry fruit esters (Figure 3). These Cabernet Sauvignon wines were also described as having higher dark fruit and red berry aromas, increased overall fruit flavour and fruit aftertaste. Moreover, from this and other studies (S. Krieger-Weber personal communication, Schmid *et al.* 2007, Bartowsky *et al.* 2008), *O. oeni* strain VP41 consistently produced red wines with enhanced berry fruity sensory characters.

Cabernet Sauvignon sourced from different viticultural regions

To investigate the viticultural region influence on MLF performance and *O. oeni* ester production, Cabernet Sauvignon fruit was sourced from four different viticultural regions in South Australia in 2008 (Clare Valley, Langhorne Creek, Padthaway and Adelaide Hills). Prior to MLF, the wines had similar alcohol content (13.9%, 14.7%, 14.4% and 14.0%, respectively) and were adjusted to pH3.45. MLF was induced with three *O. oeni* strains. Strains VP41 and Beta completely metabolised malic acid within 20-25 days, whereas strain Elios 1 needed slightly longer to complete MLF (25-37 days) (it did not complete MLF in the Clare Valley wine).

The ability of the *O. oeni* strains to modulate the fruit-related ester profile varied in each of the four respective Cabernet Sauvignon wines (Figure 4, see page 32). The proportions of red berry, blackberry and total fruity esters were little affected by MLF in the Langhorne

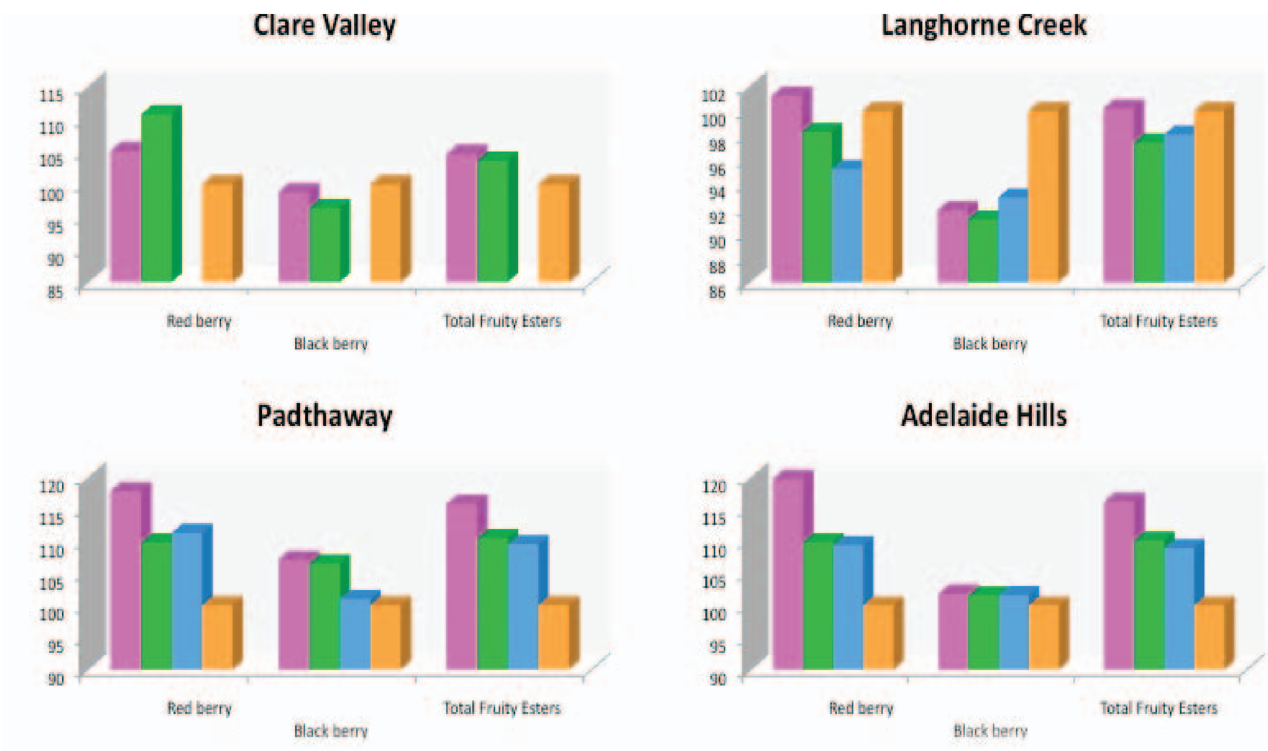

Figure 4. Sum of esters contributing to berry fruity characters (red berry, blackberry and total fruity esters), expressed as relative percentage to non-MLF (100%), in Cabernet Sauvignon wines produced from four South Australian viticultural regions (vintage 2008) following MLF induced by three *Oenococcus oeni* strains.

Creek Cabernet Sauvignon wines, whereas Adelaide Hills and Padthaway wines supported relatively greater increases in these esters with the three *O. oeni* strains. Such variation in the effects of MLF on ester concentration between viticultural regions most likely reflects regional differences in precursor content.

SUMMARY

In these MLF studies in Cabernet Sauvignon wines, the different *O. oeni* strains had varying impacts on the profile of esters that can affect the berry fruit properties of wine. In the cases where the relative total berry fruit ester content increased, an increase in sensory ratings of the fruity and berry-related terms was also generally found. This supports other reports that such ester concentrations relate to fruity-berry aromas in Cabernet Sauvignon wines and, furthermore, that different *O. oeni* strains can have a marked effect on these volatiles.

Wine chemical composition plays an important role in the metabolism of *O. oeni* during MLF. Preference by *O. oeni* to metabolise either organic acid or sugars has been shown to be wine pH dependent; at lower wine pH (below 3.5), organic acids are metabolised in preference to sugars, and conversely, at higher wine pH (over 3.7). In these studies, other *O. oeni* metabolism was influenced by wine pH; metabolism of volatile fermentation-derived compounds, including ethyl esters. In Cabernet Sauvignon, at lower wine pH there were greater increases in total berry fruity esters by certain strains compared with MLF conducted at wine pH3.7, and these differences were reflected in higher sensory ratings for fruity and berry descriptors.

Malolactic fermentation conducted in Cabernet Sauvignon fruit sourced from the same vineyard over three vintages (2006, 2008 and 2009) with three *O. oeni* strains showed that the ester production and fruity sensory attribute ratings differences for the three strains behaved similarly over the vintages. However, vineyard source of Cabernet Sauvignon grapes appeared to have a much greater influence on the ability of *O. oeni* to modulate the berry fruity ester composition.

The opportunity to use MLF to alter the sensory properties of wine is increasingly becoming a pertinent component influencing the winemaker's decision to conduct MLF in red and white wines. Bacterial metabolism during MLF in wine influences a vast pool of secondary metabolites (organic acids, diacetyl, fermentation-derived volatile compounds [esters, acetates, acids, higher alcohols], and oak compounds [when wood is used]). These studies highlight the importance of *Oenococcus oeni* strain selection and wine conditions under which MLF is conducted, to enhance the fruity and berry characters of red wine. Therefore, MLF can be a powerful winemaking tool to not only reduce wine acidity, but also to positively influence the aroma and flavour profile of the wine.

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