Early inoculation for MLF can reduce overall vinification time: laboratory and winery trials in Shiraz

Getting wine through malolactic fermentation (MLF) is an essential step in producing great wine. The importance of removing malic acid to stabilise wine is well known, and the potential for bacterial metabolism to influence aroma and flavour of the wine is increasingly appreciated. MLF can occur spontaneously by the indigenous bacterial population, after the yeast-driven alcoholic fermentation (AF) is complete, but often onset and completion of MLF is unpredictable. Inoculation with MLF bacteria takes most of the risk out of this.

Even though traditionally MLF inoculation was performed post AF, it had been noted that natural MLF can often be completed during or by the time AF had finished. Over the last 10 years, there has been renewed interest in using co-inoculation of MLF with AF as a means of stabilising wine sooner with final SO_2 additions, for maturation and bottling.

A prolonged or delayed MLF increases the risk of spoilage by other microorganisms, such as *Lactobacillus*, *Pediococcus*, acetic acid bacteria and *Brettanomyces* species. A reduction in overall vinification time saves money for the winery and, the wines are stable sooner. This allows earlier final SO_2 additions, reducing the likelihood of spoilage by microorganisms such as *Brettanomyces*. Co-inoculation or simultaneous MLF provides winemakers this opportunity.

This study was undertaken in Australian Shiraz to test co-inoculation MLF as a means of more rapidly completing wine vinification and to understand its effect on wine colour and volatile fermentation-derived composition which impact the sensory profile of wine. Several inoculation regimes were investigated including: co-inoculation, mid-AF, at pressing, and post-AF on laboratory-scale (1.5 kg fruit) and winery-scale (9 kL stainless steel tanks) trials. Shiraz grapes sourced from the Clare Valley (South Australia) were handpicked the same day for the laboratory trial, prior to machine harvesting of the remaining fruit for the winery.

Influence of MLF inoculation regime on fermentations

AF was completed for all inoculation treatments at laboratory-scale after nine days and within 10 days at winery-scale. The presence of bacteria during AF did not adversely affect the rate of sugar metabolism and ethanol production by the yeast in either trial scale.

MLF kinetics are shown in Figure 1. Co-inoculated ferments completed MLF within 20 days, and mid-AF inoculation on winery-scale completed within 21 days. At laboratory-scale, later inoculation time points required up to 45 days to complete MLF. Bacterial inoculation

at pressing or post-AF in the 9 kL tanks were slow to initiate and required reinoculation to complete MLF. Thus, early bacterial inoculation during AF reduced the overall vinification time significantly (AF+MLF): by up to six weeks in the laboratory trials, and almost three months in the winery trial.

Progress of malic acid metabolism was closely linked with the bacterial population size. This can be seen clearly in Figure 1: when bacterial numbers fell below 10^6 cfu/mL, MLF stalled, however, once the bacterial population exceeded ~5×10^6 cfu/mL, malic acid metabolism progressed well and went through to completion. The requirement for MLF reinoculation at industry-scale of pressing and post-inoculated wines was also linked to low or undetectable *O. oeni* population.

Influence of MLF inoculation regime on wine colour and phenolics

Colour is an important red wine attribute and is influenced by numerous chemical and microbial parameters. We measured colour of the Shiraz wines following different MLF inoculation regimes, as well as concentration of the phenolics components which contribute

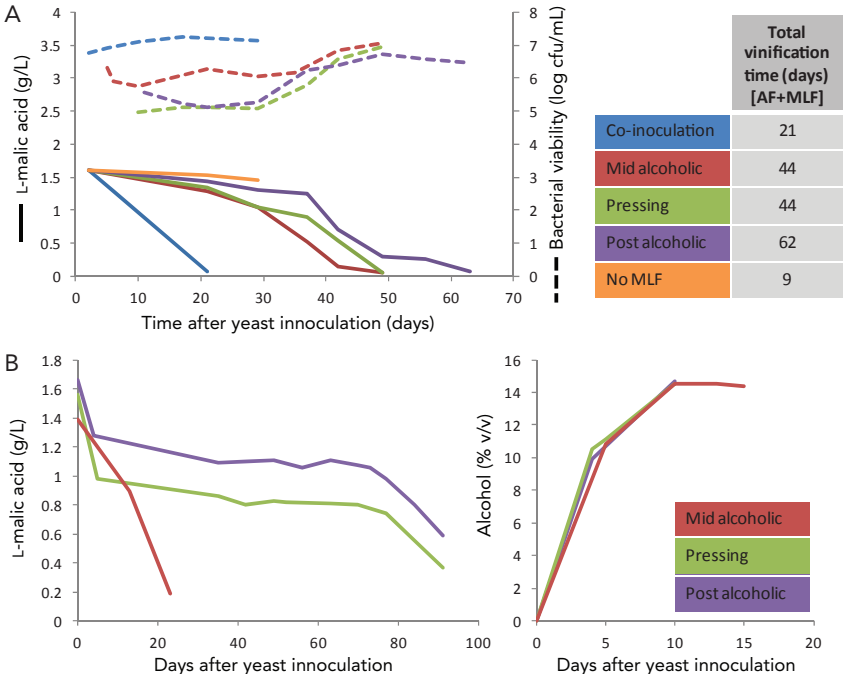

Figure 1. Effect of different timing of bacterial inoculation on the growth and degradation of L-malic acid by *Oenococcus oeni* (Viniflora oenos) during fermentation of Shiraz grape must; (A) laboratory-scale (1.5 kg fruit) (mean of triplicates) and (B) winery-scale (9 kL stainless steel tanks).

to wine colour (Figure 2). There was a slight decrease in wine colour due to MLF, however this was not influenced by MLF inoculation regime. From other studies in red wine, this decrease in wine colour is common and not usually detected in sensory studies (Massera et al. 2009, Christen and Mira de Orduna 2010).

Anthocyanins are important contributors to wine colour and decrease in concentration due to the formation of pigmented polymers as the wine ages. Malvidin-3-glucoside is the major anthocyanin found in red wine and in this study early MLF inoculation (co-inoculation and mid-AF inoculation) did not decrease malvidin-3-glucoside concentration to the same extent as the later MLF inoculation regimes. This was consistent in both small- and large-scale winemaking. The later MLF inoculation regimes (pressing and post-AF) were significantly higher in pigmented polymer concentration than those wines which did not go through MLF. The influence of MLF inoculation regime on polymerisation and co-pigmentation of anthocyanins and wine components during maturation, and the contribution to final wine colour is not clear.

Tannin content of wine contributes to various wine attributes including mouth-feel, which is linked to astringency. In our study, we observed lower concentrations of tannin in wines produced using co-inoculation. This could have an impact on astringency and mouth-feel properties of the wine, as sensory studies in Malbec wine have noted lower astringency

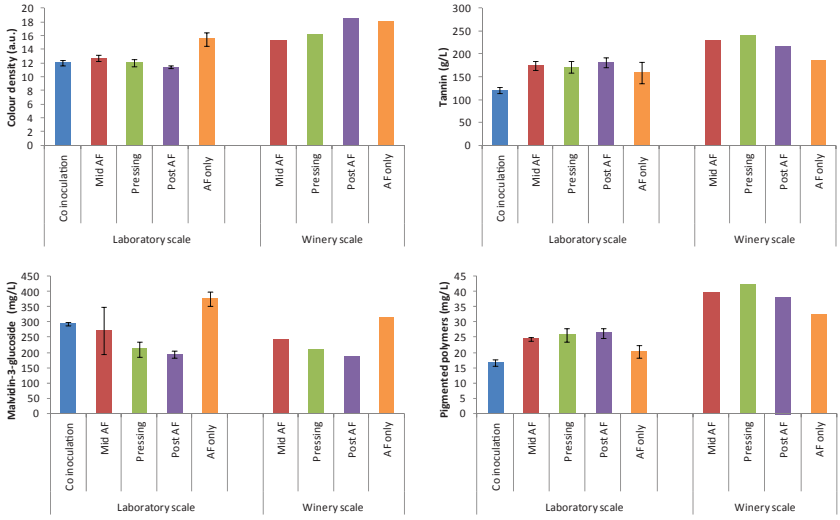

Figure 2. Wine colour density, malvidin-3-glucoside, tannin and pigmented polymers of Shiraz wines made using different MLF inoculation regimes. Vinification was conducted at laboratory-scale (1.5 kg fruit) or winery-scale (9 kL stainless tanks). Mean and standard error shown.

for wines using simultaneous (co-inoculation) MLF regime compared to sequential MLF (Massera et al. 2009).

Influence of MLF inoculation regime on volatile wine aroma compounds

Yeast and bacterial metabolism affect the pool of fermentation-derived volatile compounds (including acetate and ethyl esters, and higher alcohols) and thus consequently impact on wine aroma. We measured the concentrations of these important aroma compounds in the Shiraz wines. Figure 3 provides an overview of how the MLF inoculation regime affected the volatile fermentation-derived pool of compounds. There is a clear distinction between those wines that went through MLF and those that did not. Shiraz wines with MLF tended to have higher concentrations of compounds with fruity descriptive attributes than wines that did not go through MLF. There are also clear trends and separation of wines in relation to the timing of MLF inoculation. Small- and large-scale wines were similar in volatile fermentation-derived compounds composition, reinforcing that small-scale laboratory trials can be good model systems.

Short chain ethyl esters (up to C6) exhibit pleasant fruity, berry and green apple aroma descriptors, whereas the longer chained ethyl esters (C8-C12) retain a pleasant aroma. Changes in concentration were observed for the ethyl esters; many increased to above aroma threshold. The total concentration of ethyl esters was highest for the co-inoculation regime in this study (AWRI publication #1301). Consistent with other studies in red wines, there were variations in individual ethyl esters. Ethyl hexanoate and ethyl octanoate esters contribute to fruity aromas and were present in concentrations well above their aroma threshold (AWRI

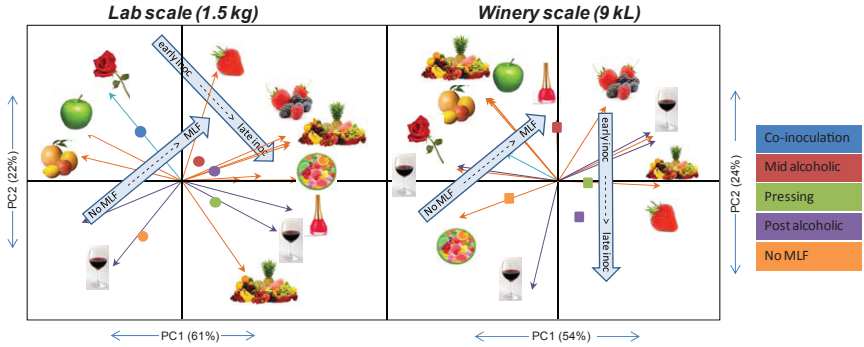

Figure 3. Principal component analysis (PCA) of volatile fermentation-derived compounds and colour components in Shiraz wine following different MLF inoculation regimes. Volatile fermentation-derived compounds with an odour activity value above 5 and colour components that are important for colour were used to generate the graphs. Treatment trends are indicated; no MLF to MLF wines, and early MLF inoculation to late inoculation regimes.
PCA vectors: Ethyl fatty esters (orange), higher alcohols (aqua) and colour components (purple). Vinification was conducted at laboratory-scale (1.5 kg fruit) or winery-scale (9 kL stainless tanks).

publication #852). In other studies from wine, co-inoculated red ferments rated higher in fruity characters than wines from sequential MLF (AWRI publication #1063, Massera et al. 2009). Together, these studies suggest that red wines produced using a co-inoculated MLF regime will most likely result in more fruity wines.

Overall, there were negligible changes in the higher alcohols following MLF compared to AF. Following MLF, acetate esters tend to decrease and this was observed in this set of Shiraz wines. Excluding ethyl acetate, the acetate esters generally decreased in our Shiraz wines post-MLF, irrespective of the inoculation regime, which concurs with studies in Aglianico and Tempranillo wines (Ugliano and Moio 2005, Canas et al. 2008).

The overall fermentation-derived secondary metabolite pool was affected by the MLF inoculation regime, with co-inoculation showing a different profile to the other inoculation regimes: mid-AF, at pressing and post-AF grouping closer together. Variations in volatile fermentation-derived compounds have been observed in other studies comparing co-inoculation with sequential MLF in Chardonnay and Shiraz wines (AWRI publication #1063).

Summary

Early bacterial inoculation greatly reduced the overall fermentation time by up to 6–12 weeks and the fermentation-derived wine volatiles profile was distinct from wines produced where bacteria were inoculated late or post-alcoholic fermentation. The rate of alcoholic fermentation was not affected by the presence of bacteria. An overall slight decrease in wine colour density observed following MLF was not influenced by the MLF inoculation regime, however, there were differences in anthocyanin and pigmented polymer composition, with co-inoculation exhibiting the most distinct profile.

This study demonstrates, with an in-depth analysis of small (1.5 kg) and large (9 kL) scales, that early or co-inoculation of yeast and bacteria in wine fermentation results in shorter total vinification time and produces sound wines. This provides the opportunity to stabilise wines more rapidly than traditional inoculation regimes permit and thereby reducing potential for microbial spoilage.

This article is a summary of a recent publication and further information can be found about the study in *Abrahamse and Bartowsky (2012) Timing of malolactic fermentation inoculation in Shiraz grape must and wine: influence on chemical composition. World Journal of Microbiology and Biotechnology* and *Early inoculation for MLF can reduce overall vinification time: laboratory and winery trials in Shiraz; Australian & New Zealand Grapegrower & Winemaker 578 [March]: 41–46, 2012.*

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For further information on co-inoculation and sequential MLF in red and white wines contact Eveline Bartowsky (eveline.bartowsky@awri.com.au).

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