Application of lysozyme in Australian winemaking

By Olivier Ciosi[1], Christophe Gerland[1], Adalberto Villa[1] and Olga Kostic[2]

INTRODUCTION

Lysozyme is an enzyme with a specific action against Gram+ bacteria. In wine, it is effective on lactic acid bacteria only and it can be used, therefore, to control malolactic fermentation (MLF), either to avoid or postpone it by addition before its onset, or to stabilise the wine after its completion.

During the 2007 vintage, six trials were conducted in South Australian wineries, testing under Australian conditions, the results that have already been obtained worldwide with lysozyme (Gerland *et al.* 1999; Gerland[a] 2006; Gerland[b] 2006).

Three different types of applications were trialled:

- Prevention of microbial spoilage by lactic acid bacteria present in a stuck wine
- Delay of malolactic fermentation in order to improve micro-oxygenation
- Stabilisation of wines after malolactic fermentation to enable a delayed SO_2 addition in order to improve polyphenol development.

AVOIDING LACTIC ACID BACTERIAL SPOILAGE WITH LYSOZYME

This application of lysozyme is common in wineries worldwide and it has already been shown to be effective (Gerbaux *et al.* 1995; Gerland *et al.* 1999).

Under warm climate conditions, with high pH wines (pH>3.6), the MLF may start before the end of alcoholic fermentation. It's even rather common in sluggish fermentations.

After total consumption of malic acid, the lactic acid bacteria may start consuming sugars and quickly produce high amounts of acetic acid, resulting in a rapid rise in volatile acidity (Fugelsang 1996).

An addition of lysozyme before the onset of the MLF or as soon as it is finished is very efficient in eliminating the population of lactic acid bacteria and in avoiding sensory faults/taints. Lysozyme's specificity against lactic acid bacteria is an advantage with respect to sulphites because lysozyme does not affect winemaking yeast (Fugelsang 1996) that may already be showing difficulty in fermenting to dryness.

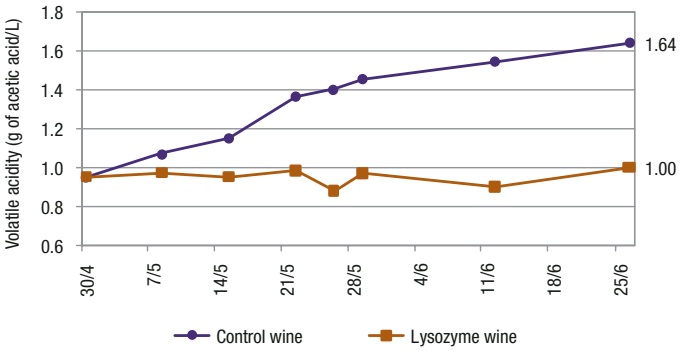

Figure 1. Volatile acidity production in the control wine and in the lysozyme-treated wine.

A stuck fermentation trial was performed in a South Australian winery with a stuck wine of 8-9g/L of residual sugars, a rather high volatile acidity (0.95g of acetic acid/L) and a high population of lactic acid bacteria. It was split in two batches with one receiving 400ppm of lysozyme. Volatile acidity evolution, lactic acid bacteria population and some other microbiological and physico-chemical parameters were recorded. Figure 1 shows the results obtained for volatile acidity and Table 1 record lactic acid bacteria population growth.

Under Australian conditions, lysozyme was very effective in preventing spoilage by lactic acid bacteria. The volatile acidity in the untreated wine (control) reached high levels in a short period of time (two months) while it is stable in the lysozyme-treated wine. Significant decrease in the population of lactic acid bacteria occurred as early as 24 hours after the addition of the lysozyme to the wine, illustrating its rapid action. No bacterial growth was reported beyond the 24 hour measurement.

In this application, lysozyme shows its advantages. Its rapid action helps to control quickly a situation affecting wine quality.

IMPROVING MICRO-OXYGENATION WITH LYSOZYME BY DELAYING MALOLACTIC FERMENTATION

Some information about the micro-oxygenation (MOX) technique is available in the boxed text on page 53, and can be useful in understanding the interest of lysozyme in improving this technique.

[1] Fordras SA, Via Molinazzo 2, 6906 Lugano, Switzerland.
[2] Fleurieu Winery Supplies, PO Box 7, Yankalilla, South Australia 5203, Australia.

Table 1. Growth of lactic acid bacteria in the control wine and in the lysozyme-treated wine (Colony Forming Unit/mL).

	01/05/07 Before lysozyme addition	02/05/07 24 hours after lysozyme addition	12/06/07 6 weeks after lysozyme addition
Control wine	$1.1x10^7$	$1.1x10^7$	$2.6x10^8$
Lysozyme-treated wine	$1.6x10^7$	60	150

Table 2. Delay of the malolactic fermentation achieved by lysozyme addition with respect to the control, and doses of oxygen added before its onset for the control and the lysozyme-treated wine.

	Delay of the onset of the malolactic fermentation	Total dose of oxygen added (mL of O_2 per L of wine)	
		Control	Lysozyme
Winery 1 (Shiraz)	3 weeks	6	11.3
Winery 2 (Cabernet Sauvignon)	1 week	1	6.5
Winery 3 (Cabernet Sauvignon)	8 weeks	2	10

In the three trials that were set up, the ability of lysozyme to delay the MLF was tested. To do so, the same wine was split into two identical tanks after the end of the alcoholic fermentation. One tank received an addition of lysozyme (at doses between 200 and 300ppm, depending mainly on the wine pH) and the other tank was un-supplemented and constituted the control.

In both tanks, MOX was started at the same rates and stopped at the onset of the MLF. The dose of oxygen added to the wine was, as a consequence, higher if the MLF was occurring later. The improvement of the MOX technique was, therefore, evaluated by comparing the total dose of oxygen that could be added (Table 2).

For the three trials, a one to eight-week delay in MLF was obtained. As a consequence, the total dose of oxygen added to the lysozyme-treated wine was always higher (from 1.8 up to six times) than the total dose of oxygen added to the control wine. The lysozyme addition has assisted in the control of the MLF so more oxygen could be added through MOX before its onset.

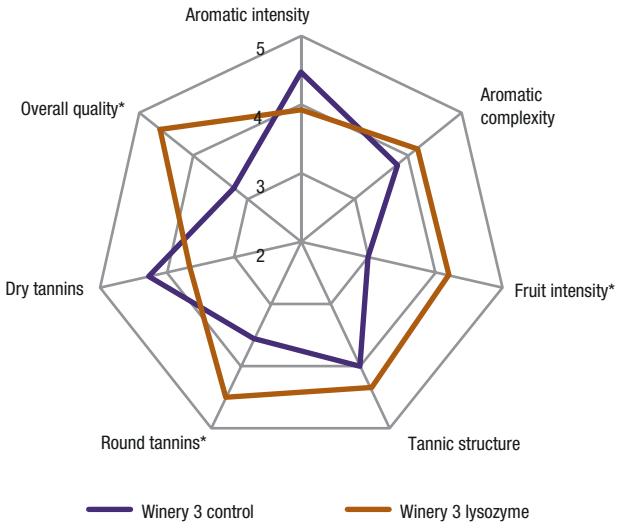

Figure 2. Sensory evaluation results for the wines from Winery 3. Sensory evaluation on 27 July 2007 (judges: 20 Australian winemakers). * Significative difference at 5% threshold.

What is micro-oxygenation?

Micro-oxygenation (MOX) is a recent technique used primarily after the alcoholic fermentation of red wines. During MOX, oxygen is added continuously and at small doses to the wine, imitating in a tank what happens in terms of oxygen diffusion during barrel ageing of wine. Different effects of this technique on the wine are reported: an increase in the colour density, a softer mouthfeel, increased fullness, decreased green/herbaceous aromas and increased ripe fruits aromas (Jones *et al.* 2004).

During MOX, the main reaction known to occur is the formation of acetaldehyde by the oxygen-catalysed oxidation of ethanol in the presence of wine phenolics. The acetaldehyde produced induces polymerisation reactions and plays a role in bridging between anthocyanins and flavonols to form anthocyanin-flavonols and flavonol-flavonols (Jones *et al.* 2004). As a consequence, the small amount of oxygen added to the wine during MOX is consumed quickly to transform the polyphenol composition via acetaldehyde. The doses commonly used are low enough to avoid an increase in the concentration of dissolved oxygen, which would otherwise lead to oxidation of the wine or microbiological spoilage. Regular tastings are essential to determine the appropriate oxygen dosage required by the wine and/or when to stop MOX. For example, a perception of acetaldehyde should alert the winemaker and the doses should be lowered because the wine is not 'consuming' the added oxygen fast enough.

It is well known that the earlier the MOX is started, especially before the MLF, the more important the impact of the technique will be on the wine profile (Crachereau *et al.* 2005; Gerland[a] 2006). Before the onset of the MLF, the phenolics are present in a higher concentration, so more reactions of polymerisation can occur, and the rates of oxygen added can be higher (from 10mL/L/month up to 120mL/L/month if the technique is used under the marc), because if excess acetaldehyde is produced at this stage, it will be consumed during MLF by the lactic acid bacteria.

It is also important to emphasise that MOX is more efficient with wines under 400 NTU, and in the absence of sulphites that inhibit the reactions occurring between acetaldehyde and phenolic compounds (Tao *et al.* 2007).

This is why the lysozyme is considered a useful tool to improve the application of MOX and better control its use in wineries. A lysozyme addition before the onset of the MLF will delay the process, giving the winemaker more time to MOX a very *'reactive wine'*.

In the case of Winery 3, MOX was stopped after two weeks because the winemakers concluded that the ideal sensory profile had been reached (Figure 2). The moment when MOX should be stopped is still a crucial issue for the results obtained with this technique, but the use of lysozyme aids winemakers in controlling their best period of intervention, which is before MLF.

Figure 2 shows that the lysozyme-treated wine presents more fruit intensity on the nose and rounder tannins on the palate. The overall quality is judged superior than the control wine.

STABILISING WINE AFTER MALOLACTIC FERMENTATION WITH LYSOZYME THEREBY AIDING POLYPHENOL DEVELOPMENT BY DELAYING SO_2 ADDITION

One last application of lysozyme was tested in two wineries on a Pinot Noir and a Shiraz wine. After MLF, each wine was split into two batches, one control receiving the usual dose of sulphites practiced in the winery (between 50 and 70ppm) and one receiving an addition of lysozyme (250ppm). This last batch was left for a few weeks in absence of SO_2 before being stabilised by sulphites at the same dose used in the control. During the period of the trial, the wine was put into barrels for ageing (lots of five to six barrels of similar age and identical coopers).

In absence of SO_2 the reactions between polyphenols and oxygen are not inhibited (Tao *et al.* 2007) and the phenolic profile of the wine is modified as is seen during MOX (see boxed text about micro-oxygenation, page 53). What was tested in these two trials was the effect of a delayed sulphite addition on the phenolic profile of the wine. Lysozyme is used here as an alternative to SO_2 for stabilising the wine with respect to lactic acid bacteria.

Regular tasting and microbiological analyses were performed to avoid oxidation/or microbiological spoilage by *Brettanomyces* or acetic bacteria. Some results of the data obtained are presented in the next paragraph.

The effect of this technique on the wine's profile was noted to be significant in terms of the dryness of the mouthfeel (*'Dry tannins'* descriptor), a property found to be much higher in the case of classical SO_2 stabilisation. The stabilisation of the wine with lysozyme allowed a period of an absence of SO_2 after the MLF that had an evident influence on the polyphenols development.

This technique can also be of interest to save colour of wine made with low coloured grapes. It is used in Burgundy on Pinot Noir, where it allows an increase in colour and softening of tannins (Gerbaux *et al.* 2003) with respect to a direct SO_2 addition.

MANAGING *BRETTANOMYCES* RISK IN WINES WITHOUT SO_2

This topic alone could be the subject of another article but as the techniques presented here raise questions about winemaking in the absence of sulphites in some parts of the process, we will stress an important point: the risk of microbiological spoilage for wines that have no SO_2 can be easily managed through simple microbiological testing.

The Sniff'Brett® test (Intelli'oeno, available from Fleurieu Winery Supplies), allowed us to easily check the *Brettanomyces* population in the wines and provided us with a rapid result to inform the participating wineries of the level of risk.

Our results showed an absence of *Brettanomyces*, as no growth was detected for most of the trials, even in absence of SO_2. In the instances where *Brettanomyces* was present, the population could be lowered by simple interventions like starting the MLF through inoculation of commercial bacterial strains, racking and sulphite addition, in order to avoid volatile phenol production. The simple microbiological testing (Sniff'Brett®

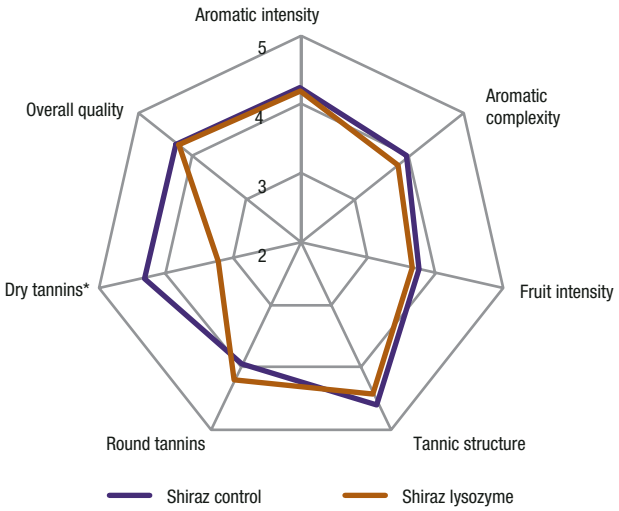

Figure 3. Tasting notes of the Shiraz wines (control and lysozyme-treated wines). The lysozyme-treated wine was left five weeks without SO_2 after the end of malolactic fermentation. Sensory evaluation on 27 July 2007 (judges: 20 Australian winemakers).
* Significative difference at 5% threshold.

detection media) assisted us in making the decision as to whether it was safe or not to go on with the trial without the presence of SO_2 or if the wines required stabilisation by a sulphite addition.

CONCLUSION

The trials conducted under Australian conditions confirm the results previously obtained worldwide with lysozyme.

Lysozyme is not only a problem-solving tool for application when the risk of lactic acid bacteria spoilage is high, but can be of interest in improving wine quality in both traditional and modern winemaking.

We present here a few examples (micro-oxygenation and ageing) but other applications can be considered:

- white winemaking with ageing on the lees without MLF
- white winemaking without MLF with delay of the SO_2 stabilisation to avoid reductive aromas
- sparkling winemaking without MLF
- red winemaking with a delay in MLF (traditional Pinot Noir winemaking in Burgundy)
- wines with reduced doses of sulphites and some examples of wines without SO_2.

For more information about the various applications of lysozyme in winemaking, visit the interactive website www.lysoclub.com, where you will find advice on lysozyme utilisation, dose calculation (based on the parameters of your wine), certification documentation and bibliography of all research.

ACKNOWLEDGEMENT

This study was undertaken with the technical support of Dr Vladimir Jiranek and Dr Paul Grbin of the University of Adelaide.

REFERENCES

Crachereau, J-C.; Gervais, J-P.; Pilatte, E. and Cauchy-Alvin, B. (2005) Gestion de la micro-oxygénation et de la fermentation malolactique des vins rouges. Revue des Œnologues, n°116, July, pp 24-28.

Fugelsang, K.C. (1996) Wine microbiology, . The Chapman & Hall Enology Library. pp 29, 153.

Gerbaux, V.; Villa, A.; Monamy, C. and Bertrand, A. (1999) Utilisation du lysozyme pour inhiber la fermentation malolactique et pour stabiliser les vins après fermentation malolactique. Bulletin de l'OIV, 819-820, pp 352-373.

Gerbaux, V.; Briffox, C.; Villa, A.; Lagarde, G. (2003) The impact of lysozyme on the colour of red wines. Vinidea.net – Wine Internet Technical Journal, n°4. www.infowine.com

Gerland, C.; Gerbaux, V. and Villa, A. (1999) Le lysozyme, nouvel outil biotechnologique pour maîtriser les bactéries lactiques. Revue des Œnologues, n°93S, pp 44-46.

[a]Gerland, C. (2006) Optimisation de la micro-oxygénation grâce au lysozyme. Revue des Œnologues, n°120, July, pp 45-46.

[b]Gerland, C. (2006) Gestion des bactéries lactiques et succès commercial. Conférence Vinitech-Vinidea, November.

Jones, P.R.; Kwiatkowski, M.J.; Skouroumounis, G.K.; Francis, L.; Lattey, K.L.; Waters, E.; Pretorius, I. S. and Høj, P. B. (2004) Exposure of red wine to oxygen post-fermentation. The Australian and New-Zealand Wine Industry Journal, May/June, Vol. 19, number 3. pp 17-24.

Tao, J.; Dikes, S.I. and Kilmartin, P.A. (2007) Effect of SO_2 concentration on polyphenol development during red wine micro-oxygenation. J. Agric. Food Chem., 55, pp 6104-6109.