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5.13 What is lysozyme and why is it used in Winemaking

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Lysozyme is an enzyme that is extracted from the egg white of hens but also exists in mammalian milk, tears and saliva. For many years, it has been used as a natural antimicrobial and antiviral in the food and pharmaceutical industries (Davidson and Zivanovic 2003). It can be used in winemaking to prevent microbial spoilage by *gram positive* bacteria, delay malolactic fermentation (MLF) or delay sulfur dioxide (SO_2) additions after MLF is complete. It is usually effective within 1-3 days but does not provide a lasting protection in red wines. In contrast, lysozyme has been observed to remain active for up to six months in white wines (Bartowsky *et al.* 2004). Lysozyme cannot replace sulfur dioxide (SO_2) because it will not prevent infections by other spoilage organisms such as *Acetobacter* or *Brettanomyces. It* is very important to monitor the wine for VA regularly and for the presence of other spoilage microbes. It will not affect desirable yeasts either. Lysozyme can be used in organic wines in the USA at concentrations up to 500 mg/L.

In juices, must and wines, the main gram-positive bacteria are lactic acid bacteria *(Pediococcus*, *Lactobacillus* and *Oenoccocus).* We use *Oenococcus* in our wines to convert malic acid into lactic acid (MLF) to prevent this microbial action happening in an uncontrolled way at an undesirable time (e.g., after bottling), to lower the pH and or to change the flavor profile of our wine (e.g., to introduce buttery flavors). On the other hand, we do not want *Lactobacillus* or *Pediococcus* to carry out MLF because they produce undesirable compounds such as volatile acidity (VA). Problems can even arise when our wines contain *Oenococcus* if MLF finishes before alcoholic fermentation. At the end of MLF, all lactic acid bacteria may begin to consume sugars and produce high levels of VA, ruining the wine.

Spoilage of juice or musts due to lactic acid bacteria can happen when we have diseased grapes, unsanitary conditions or a stuck or sluggish alcoholic fermentation. Once alcoholic fermentation commences, we cannot control the bacterial population with SO_2 because it will also inhibit the production of alcohol by yeast. On the other hand, lysozyme additions will eliminate the lactic bacteria population without inhibiting the yeast. Hence, the addition of lysozyme can prevent unwanted increases in VA before or during alcoholic fermentation because it will not inhibit yeasts. Nevertheless, prevention is always better than cure and can be achieved by sorting out diseased fruit, keeping the winery clean and sanitizing equipment before use.

Immediately monitoring the microbial population in juices or in musts from diseased or low quality grapes (by microscopic evaluation or other tests) will indicate whether a lysozyme addition may be warranted. Tracking the rate of decrease of glucose/fructose concentration of fermenting juices or musts will

I. Potential users of lysozyme need to check the legal requirements for its use in their region.

alert us to a sluggish or stuck fermentation. If this occurs we need to establish if lactic acid bacteria are present so that we know that the addition of lysozyme may be beneficial.

Lysozyme additions may be useful when we wish to delay MLF (some winemakers like to delay MLF of Pinot noir for 3-6 weeks after alcoholic fermentation). Adding lysozyme at this stage may help prevent the onset of MLF and infection by undesirable lactic acid bacteria.

If lysozyme has been added to a red wine, some will react with phenols and may produce lees which can be removed by racking. There also may be some color loss in wines made from varieties that have potentially low color, such as Pinot noir (Bartowsky *et al.* 2004). In white and rosé wines, the lysozyme may contribute to protein hazes (Bartowsky *et al.* 2004, Weber *et al.* 2009) and so *treated* wines may require bentonite fining before bottling. Fining trials would need to be run to ascertain how much bentonite would be required but the presence of lysozyme has been shown to at least double the rate of bentonite required to heat stabilize Riesling and Pinot blanc (Weber *et al.* 2009).

Lysozyme can be purchased as a solid or as a liquid and could cost from $31-185 per 1000 L juice or wine, depending on the rate applied (150-500 mg/L), the form used and whether a discount was offered for purchasing larger quantities. The maximum rate of 500 mg/L is recommended to inhibit unwanted malolactic bacteria and/or prevent MLF, while 250-300 mg/L is considered sufficient to delay the onset of MLF. Less may be required in white and rosé wines because there are far less phenols present to potentially denature the enzyme.

Lysozyme is an allergen and the EU has decided to require declaring its presence in wine on the label. Estimates of the percentage of the population that suffers from allergic reactions to egg protein appear to be 0.09% but the role that lysozyme specifically plays is less clear (laconelli *et al.* 2008). White wines treated with the enzyme have been found to contain concentrations harmful to allergic consumers. Without bentonite fining, 34-73% of lysozyme was found to remain in Riesling, Pinot blanc and Pinot gris wines and approximately 10% in a Dornfelder red wine. On the other hand, bentonite fining decreased the amount of lysozme to negligible concentrations (Weber *et al.* 2009).

References and further reading

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