GLUTATHIONE AND ITS APPLICATION IN WINEMAKING

What is glutathione?

Glutathione (L-γ-glutamyl-L-cysteinyl-glycine) is a tripeptide which contains three constitutive amino acids: glutamate, cysteine and glycine.

In must, wine or even yeast, glutathione can be found under its reduced (GSH) or oxidized form (GSSG), the later being two molecules of glutathione linked by a sulfide bridge.

It is present in plants and foods, and fruits like grapes. It is also the most abundant nonproteic sulphur compound found in most living organisms including the wine yeast Saccharomyces cerevisiae.

Why is it important in wine?

Glutathione (GSH) is important in wine because it has the ability to scavenge ortho-quinones, main protagonists of color browning and aroma loss due to oxidation mechanisms. Because it has a very low oxydoreduction potential ($E'_o=-250$ mV at pH 7.0 ; $E'_o=-40$ mV at pH 3.0), it can act as a strong buffer in many cellular oxydoreduction reactions. It has been known for years that it is a more potent anti-oxidant than ascorbic acid ($E'_o=+60$ mV at pH 7.0 ; $E'_o=+267$ mV at pH 3.0). It then plays a critical role in preventing the oxidation of must phenols as it can react via its –SH group with caftaric acid – one of the most susceptible phenols to oxidation in musts and generate Grape Reaction Product (GRP) which is a stable and colorless compound (Moutounet et al, 2001).

Notably, this mechanism has been shown for GSH and not for the other compounds present in must or in yeast, and possessing SH-group (such as cystein or glutamyl-cystein for instance).

GSH can also compete with several thiols (aromatic compounds such as 3-mercapto-hexanol (3MH), its acetate 3-mercapto-hexanol acetate (3MH-A) and 4-methyl-mercapto-pentanone (4MMP)) present in wines under the form of precursors or aromatic molecules, for o-quinones thus protecting certain wine aromas (Dubourdieu et al, 2003). The effect of GSH on wine as a natural anti-oxidant for the preservation of wine aroma and colour is well understood now.

How can we influence the glutathione levels in must and wine?

Having high levels of glutathione in wine is then important for the preservation of aroma and color. The level of glutathione can vary in must as it is based on grape varietals, viticultural practices and the winemaking practices.

As glutathione can’t be added to must or wines, the use of specific inactivated yeast rich in glutathione (GSH-rich SIY such as Optiwhite®, OptiMUM White® and Booster blanc®) becomes an interesting natural alternative to optimize the quality of wines. GSH-rich SIY will also vary in the quantities and quality of GSH that they contain and how they are measured to reflect that quantity. The most important GSH that is needed to be active must be in the reduced form as this is the active anti-oxidant form.

Among Lallemand portfolio, specific wine yeast are chosen for the production of OptiWhite®, OptiMUM White® and Booster Blanc® (patent N°WO/2005/080543) and the process from yeast multiplication to inactivation and drying is also adapted in order to get a high content of soluble reduced glutathione in the corresponding biomass. The ability of the inactivated yeast to release GSH in the media after its addition is also an important criteria.
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Consequently, the criteria to have the best GSH-rich SIY are:

- “True GSH” content measured
- GSH has to be quickly released in the medium
- GSH has to be under its soluble & reduced form → the only form that can be active and efficient towards oxidation mechanisms

Some parameters have to be considered when using GSH-rich SIY

Yeast nitrogen requirement:

GSH being a tripeptide is a nitrogen source for yeast. Thus, in case of must nitrogen depletion, yeast can use GSH for its nutrition. It is therefore very important to manage the fermentation and supply sufficient balanced nitrogen nutrition in order to avoid the loss of GSH by it being taken up by the yeast as a nitrogen source. This is all the more important if the selected yeast is known for its high nitrogen requirements. In that situation, a careful monitoring of the alcoholic fermentation needs to be done.

Timing of addition:

The timing of addition of the GSH-rich SIY is also important. Based on studies done by Aguera et al. (2012) and Kritzinger et al. (2012), the best time to add a tool such as OptiMUM White®, which has the highest GSH content, is in the early stages of alcoholic fermentation. More on this issue will be discussed in this Wine Expert.

A WORD FROM OUR EXPERT

GSH-rich SIY appear to be an interesting tool to provide glutathione to the must in order to help protection against oxidation. Literature on the topic and our own research showed differences in the amounts of GSH released by different SIYs.

These differences seem to be linked to differences in the manufacturing processes among the preparations. Our results illustrate the variation that exists among GSH-rich SIY in terms of GSH content. Moreover, it shows the importance to differentiate between GSH and total GSH contents since it is the reduced form that is the active antioxidant in wine.

From this data is can be deducted that the production process of (Optimum White®) is optimized to such an extent that it contains a considerably higher GSH content. This product could possibly be more efficient in reducing the oxidation phenomena in wines when compared to the other GSH-rich SIY in this study.

Dr Wessel du Toit from the Department of Viticulture and Oenology, Stellenbosch University (South Africa), is doing research on wine anti-oxidant and oxidation in wine. He has collaborated extensively with Lallemand in the past. He has published a number of articles on the effect of oxygen in wine and is also teaching this part of Oenology.
Many SIY products on the market claim to be rich in GSH. What does it really mean? Are we talking about the same thing all the time?

It is important to understand that the value associated with different GSH-rich SIYs is related to how the GSH content is measured. If it is measured by the classical chemical reaction (Figure 2), it will quantify all molecules with a –SH group released by the SIY and not specifically GSH under its reduced form. The result is expressed in terms of “GSH equivalent” and not “true GSH”.

The best way to obtain a real measurement of true glutathione is by using a specific HPLC/UPLC method which will reveal the content of reduced GSH, which is, as mentioned before, the active form of GSH for must and wine color and aroma preservation. In parallel, the oxidized glutathione (GSSG) and other compounds such as cysteine and glutamyl-cysteine can be precisely measured. An illustration of the results brought by this method is shown in figure 3. This is the method used to measure the GSH in all the GSH-rich SIYs for products such as OptiWhite®, OptiMUM White® and Booster Blanc®.

Figure 4 shows the results of several GSH-rich SIY’s analysis and the variations between the methods of analysis. For example, using the classical chemical method (which measures all –SH molecules and not only the active reduced GSH) and using the HPLC methods (which measures only the active reduced GSH), lead to a different interpretation. OptiWhite® and Booster Blanc® present the same profile of GSH content whereas OptiMUM White®, due to the process optimization presents the highest level of true GSH by far which means that this product will be the most efficient to protect color and aroma in must and wine.

We can also note that the product Y has the highest level of “GSH equivalent” but is also the one with the highest content in cysteine, which might not be the most interesting and suitable compound regarding its impact on wine quality.
In a study done by the Department of Viticulture and Oenology, of Stellenbosch University (Kritzinger et al., 2012), UPLC analysis revealed the total, reduced and oxidized GSH contents released into a wine model solution by these different GSH-rich SIYs.

We observe in Figure 5 that OptiMUM White® released by far the highest level of reduced GSH released into the wine model solution. The 4 other products presented the similar amount of reduced GSH released in the wine model solution. However, one of the 3 other products (Product 3) presents a high level of total GSH but most of it being under oxidized form (GSSG) which is not efficient in must and wine. These results clearly show that OptiMUM White® released the highest quantity of glutathione necessary to protect wine quality.

Figure 5: Reduced (GSH), oxidized (GSSG) and total glutathione (reduced + oxidized GSH) content released by various GSH-rich SIY a wine-model solution.

Jean-Michel Salmon has followed a classical university education training through the obtention of a Master of Sciences in Industrial and Food Biochemistry (ENSIA-University Paris VII, 1981). After a doctorate in microbiology held in 1986 (University of Montpellier), and a post doc at the CSIC in Madrid (C. Gancedo’s lab), he joined as a research scientist the microbiology research team at INRA in Montpellier. Since 2003, he was Research Director in this institute, and since 2 years was in charge of the direction of the INRA’s experimental unit of Pech Rouge. His main research topic was the physiology of yeasts (Saccharomyces cerevisiae), and more precisely the study of its interactions with oxygen during alcoholic fermentation and wine ageing. Moreover he has developed a practical knowledge on the use of yeast mixed cultures during alcoholic fermentation. His scientific production relies on 76 peer-reviewed papers, 38 wine-related professional papers, 10 book chapters, 50 international oral conferences, 37 posters in congress and 4 patents.

We’ve realized research work in the frame of the development of the application of GSH-rich SIY in winemaking. Namely we’ve focused on the determination of the best timing of addition and the impact of such a practice on thiols and other aromatic compounds.

Our results show the positive impact of the addition of GSH-rich SIY on color stability and volatile thiols preservation during aging, provided this addition is done at the beginning of AF. We could also notice that the efficiency of the treatment is all the more important that the alcoholic fermentation is well managed in terms of yeast choice and nutrition aspects (Aguera et al, 2012).
2.1 Fermentation management

As previously indicated, when using GSH-rich SIY such as OptiMUM White®, a proper fermentation management is crucial, as yeast with high nitrogen requirement can use the amino acids of GSH-rich SIY as a nitrogen source, and therefore diminishes the impact of the inactivated yeast releasing the GSH in wine. The results of figure 6 (Aguera et al., 2012) show the impact of the addition of OptiMUM White® in a Syrah and a Grenache rosé wine, where Yeast B, with a high N demand, shows the least impact on the level of thiols (3MH, 3MHA) found in the wine from the addition of OptiMUM White®. The other yeasts, with low to medium nitrogen demand, do show an important impact on the thiols concentration found in the wine compared to the wine which had not received OptiMUM White®.

2.2 Timing of addition

In a study done by Aguera et al. (2012), the impact of the timing of addition of the GSH-rich SIY OptiMUM White® was studied. OptiMUM White® was added during yeast inoculation, at the beginning of fermentation (43 hrs during AF) and at the end of AF. The results (Figure 7) showed that during accelerated aging, there was a positive impact on thiol preservation when OptiMUM White® was added at the beginning of fermentation. Further trials were done in Grenache and Syrah for rosé wines in cellars, and after one year of aging, the wines were analyzed and found to have gains in varietal thiols of 3MH and 3MHA that were significant when again OptiMUM White® was added at the beginning of fermentation.
2.3 The impact on aromas

Many studies have shown the impact of glutathione on various aromas such as terpenes, esters and volatile thiols (Fragasso et al. 2010, Andújar-Ortiz et al. 2010, Curtin, 2009). Some results are shown in figure 8, where different aromas compounds (esters and terpenes), were measured in Roupeiro et Rabo de Ovelha varietal wines (Portugal) treated with OptiWhite® and Optimum White®, and compared to a control. The wines treated with OptiMUM White® had significantly more esters and terpenes than the control and the wine treated with OptiWhite®.

Figure 8. Analysis of variations (%) of aroma compounds from Roupeiro et Rabo de Ovelha wines (Portugal) treated with OptiWhite® (red) and OptiMUM White® (green) versus the control without addition (from Aguera et al. 2012).

Theses differences in aroma compounds composition resulted in a sensory impact that is shown in figure 9 where the same wine treated with OptiMUM White® had significantly more positive aromas and gustative balance.

2.4. The impact on longevity of aromas

Knowing that OptiMUM White® has high levels of GSH in its most active form is reflected in the results obtained with this product in wines, especially in terms of longevity. For example, Figure 10 shows the results of a trial done in Sauvignon blanc from France, where the wines have, after one year of aging, higher levels of the aromatic thiols such 3-mercaptotetanol (3MH) and its acetate (3MHA) compared to the control with no SIY enriched in GSH.

Figure 10. The levels of thiols (3MH and 3MHA) in Sauvignon Blanc treated with Optimum White® one year after bottling.
A QUICK SUMMARY

GSH-rich SIY are natural winemaking tools that can be used to favor and improve aroma intensity and longevity as well, and protect the color of white and rosé wines. OptiMUM White® is a new specific inactivated yeast rich in glutathione that benefits from a new optimized production process that enhances the reduced glutathione availability. It contains the highest level of true active and efficient form (reduced form) of GSH. This product will have positive impact on color, wine thiols content, wine esters and terpenes and on the sensory properties; as well as an impact on the preservation of aromatic compounds during aging.

Our next topic: “Co-Inoculation of Selected Wine Bacteria”