Beyond glutathione
Protecting wine aroma and colour using a unique glutathione-rich inactivated yeast

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Wine oxidation is a concern for every winemaker. Traditionally, sulfur dioxide is used to manage oxidation, but with increasing interest in reducing the use of SO_2, natural alternatives are being sought. Glutathione, a tripeptide which is formed through the natural metabolism of yeast, is a potent antioxidant. Inactivated yeast rich in glutathione offer a non-chemical means to manage grape juice oxidation.

OXIDATION MECHANISMS THROUGHOUT WINEMAKING

Oxidation in oenology is a big word that covers many different dimensions, from the colour of a wine through to its aromatic composition to its longevity. It could be defined as "all the chemical or physical reactions happening in a must or in a wine in the presence of oxygen". Managing oxygen is still challenging, so most of the solutions to reduce oxidation are dedicated to neutralising or minimising the first oxidation product, the quinones that are oxidised polyphenols.

Indeed, quinones in wine are involved in many reactions leading to the accumulation of aldehydes (source of off-flavours), the first steps of wine browning and are involved in the trapping of varietal thiols (Figure 1).

Traditionally, sulfites (such as SO_2) are used as the main antioxidant compound to reduce quinones back to polyphenols, but other compounds naturally present in wine are also able to trap quinones and thus minimise the oxidative damage. One of the most abundant and known of these compounds is glutathione.

GLUTATHIONE IN WINEMAKING

Glutathione is a tripeptide, which contains three constitutive amino acids: glutamate,

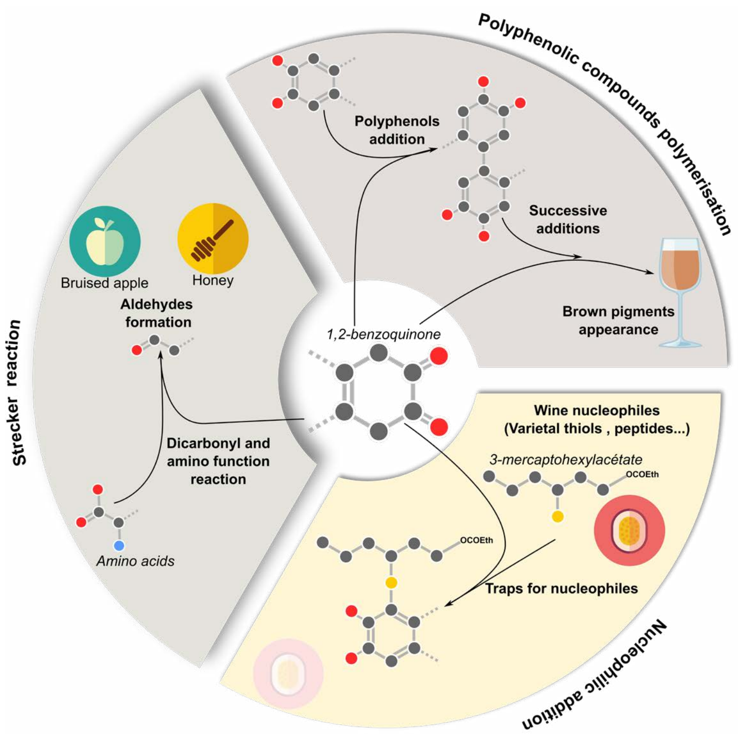

Figure 1. Quinones at the centre of the main oxidative damage to the wine. The objective in using inactivated yeast naturally rich in glutathione is to compete with aroma compounds that could bind to quinone, notably nucleophilic addition, thus leaving the aroma compounds free to contribute to a wine's sensory qualities and minimise wine oxidation (adapted from Oliveira *et al*. 2011).

IN **BRIEF**

- With the growing trend towards reduced use of sulfur dioxide in winemaking, other methods of managing oxidation are being sought.
- In its reduced form, glutathione, a tripeptide, can scavenge quinones responsible for browning and aroma loss due to oxidation mechanisms.
- Although pure glutathione cannot be used in winemaking, inactivated yeast rich in reduced glutathione are approved for use.
- A new inactivated yeast naturally enriched with reduced glutathione was trialled on rosé for its effectiveness against oxidation and aroma compound loss.

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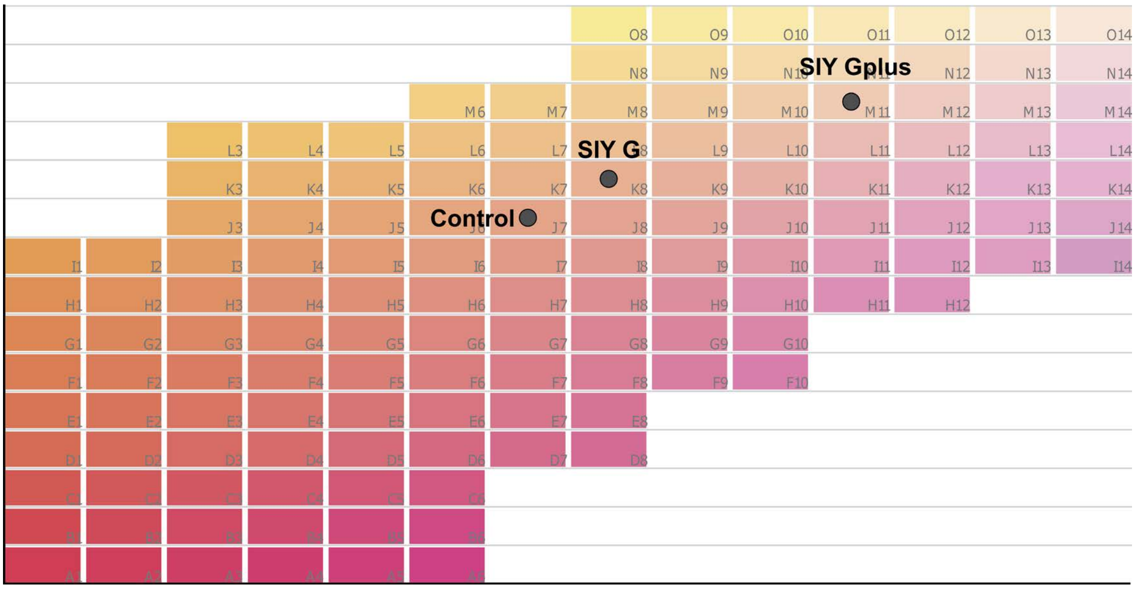

Figure 2. A comparative trial of the application of different inactivated yeast (SIY G, SIY GPlus [marketed as Glutastar]) at the time of clarification of a Syrah-Grenache rosé must (Provence, France).

cysteine and glycine. It is formed through the natural metabolism of yeast. In wine, glutathione can be present in a reduced (GSH) or oxidided form (GSSG). Glutathione is important in winemaking in its reduced form because it can scavenge quinones responsible for browning and aroma loss due to oxidation mechanisms (Lavigne *et al*. 2007). It is well known that GSH is a more potent antioxidant than ascorbic acid (Antoce *et al*. 2016), but there are still some uncertainties when applied in commercial winemaking (Schmidt *et al*. 2020). In addition, pure glutathione cannot be used in winemaking according to FSANZ and OIV regulations.

However, inactivated yeast rich in GSH are approved for use in winemaking. The International Organisation of Vine and Wine (OIV) has recently adopted and incorporated a monograph (OIV-OENO 603-2018) on inactivated yeasts containing glutathione into the international oenological codex. These yeasts are also authorised in the EU (EU 2019/934).

THE GENESIS OF A SPECIFIC INACTIVATED YEAST RICH IN GLUTATHIONE

Empirically, ageing on lees produces wines with better oxidative stability. This is attributed to the oxygen consumption and the release by yeast of many beneficial compounds (e.g., lipids, polysaccharides, peptides). The use of inactivated yeast can mimic ageing on lees since they can exhibit antioxidant properties without microbiological risks.

Several studies have shown that inactivated yeast containing glutathione are more efficient in preventing oxidation than GSH (Andújar-Ortiz *et al*. 2010, 2014, Comuzzo *et al*. 2015, Rodríguez-Bencomo *et al*. 2016, Pozo-Bayon *et al*. 2009, Rodríguez-Bencomo *et al*. 2014, Gabrielli *et al*. 2017). More recently, it has been confirmed that inactivated yeast release a large amount of peptides with antioxidant potential which co-accumulate with the glutathione (Bahut *et al*. 2019). Thus, it is apparent that it is a combination of the GSH with peptides and nucleophilic components present in the inactivated yeast that are protecting wine aroma compounds.

A specific inactivated yeast (SIY) that has a high glutathione content is produced when a specific wine yeast strain is subjected to a process that ensures the synthesis of glutathione by the yeast and its accumulation in its reduced form in the yeast biomass before inactivation. A new specific inactivated yeast (SIY GPlus) with innovative metabolic characteristics has been developed. The large number and diversity of compounds released by the SIY have numerous winemaking implications, in particular on oxidative stability. This natural oenological tool is an alternative to traditional antioxidants.

IMPACT ON WINE QUALITY

Protection of wine colour

A comparative trial was set up at the experimental winery of the Centre du Rosé (Provence, France) on a pilot scale using a Syrah-Grenache rosé must obtained by direct pressing to investigate the impact of SIY GPlus on rosé wine colour. Different must treatment strategies were evaluated at the time of clarification. The addition (30g/hL) of SIY GPlus was compared with a traditional specific inactivated yeast rich in glutathione (SIY G) and an untreated control. Wines were made under standard conditions and the different treatments were monitored until bottling. In particular, the colour was evaluated, and the results of the analyses are shown on the rosé wine colour chart in Figure 2. The lowest orange shade, considered to be higher quality, was obtained with the early treatment of SIY GPlus.

Protection of aromatic compounds

The positive impact of SIY GPlus was also observed on wine aromas. Numerous trials were carried out during white winemaking,

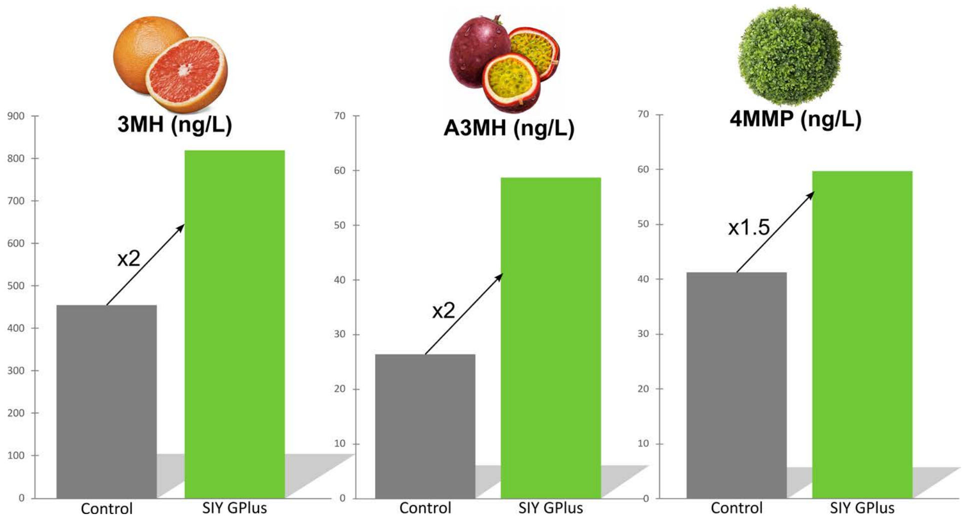

Figure 3. Application of SIY GPlus (marketed as Glutastar) before a cold juice stabulation of eight days at 4°C compared to a control with no addition – thiol analysis in bottled wines (Sauvignon Blanc, Loire Valley, France).

notably on Sauvignon Blanc. This grape variety is ideal for studying the oxidative stability of aromas, as thiols are key markers of oxidation.

One trial conducted in the Loire Valley (France) evaluated the impact of SIY GPlus (30g/hL) added to a Sauvignon Blanc must before a cold juice stabulation of eight days at 4°C. The winemaking process was the same and the aromatic compounds were analysed in the bottled wine. The results, presented in Figure 3, demonstrate the protective effect of SIY GPlus with varietal thiol levels higher than in the untreated control.

Another trial conducted on a Sauvignon Blanc (Loire Valley, France) evaluated the impact of the addition of SIY GPlus (30g/hL) to free run juice at the press. The aroma index measured in the bottled wines based on Odour Activity Value (aroma compound concentration relative to aroma compound sensory threshold) (Figure 4, see page 18) shows that SIY GPlus provided better preservation of aromatic compounds and a higher vegetal thiolic perception as well as more citrus compared to the control.

Another trial with SIY GPlus was undertaken in Chardonnay at the Institut Universitaire de la Vigne et du Vin (IUVV, Burgundy, France) in the context of decreasing the use of SO_2. Grapes were harvested by hand and 2.5g/hL of SO_2 was added in the free run juice at the press. After a cold settling (24 hours at 15°C), 2.5g/hL of SO_2 addition ('Full SO_2') was compared to SIY GPlus at 30g/hL ('Half SO_2 + SIY Gplus') and a control with a half dose of SO_2 ('Half SO_2'). The alcoholic and malolactic fermentations were managed in the same way and a sensorial analysis was conducted on bottled wines. As presented in Figure 5 (see page 18), the wine with half SO_2 and SIY GPlus addition was significantly less reductive, less vegetal and exhibited more fruit and floral aromas. In addition, the total SO_2 content in the final wines was divided by two for half SO_2 treatments (19mg/L) compared to full SO_2 (41mg/L).

SCIENTIFIC EVIDENCE BEHIND SPECIFIC INACTIVATED YEAST

Metabolomic studies of different types of inactivated yeast to understand the mechanisms of protection

In the past, traditional chemistry and analytical methods were used to detect and quantify various groups of compounds such as sugars, proteins and lipids. More recently, new methods have been developed to target all the compounds present in a yeast cell or matrix, rather than a single target compound. Metabolomics makes possible the simultaneous analysis of all metabolites present and, subsequently, can be classified according to their element composition (C, H, O, S and N) and chemical families (sugars, proteins, lipids) (Figure 6 B and C, see page 18). Metabolomic analysis has been performed in collaboration with the PCAV team at the Institute of Wine and Vine, Dijon, France.

Metabolomics is a powerful tool that finds relationships between chemical composition and specific activity, such as antioxidant activity in our example. First, metabolomics analyses showed vast differences between various types of inactivated yeast based on both the yeast strains used and the culture conditions during production. SIY GPlus, marketed under the name Glutastar, exhibited an exceptional abundance of CHONS compounds. Further research showed that these compounds are related to peptides containing cysteine residues. These peptides are potent antioxidants as they have a free sulfhydryl function, such as glutathione.

The traditional DPPH antioxidant assay has been recently modified to measure antioxidant capacity in a wine like medium (Romanet *et al*. 2019). This assay has been applied to different samples of inactivated yeasts (Figure 6A, see page 18) and it showed two main results. First, the

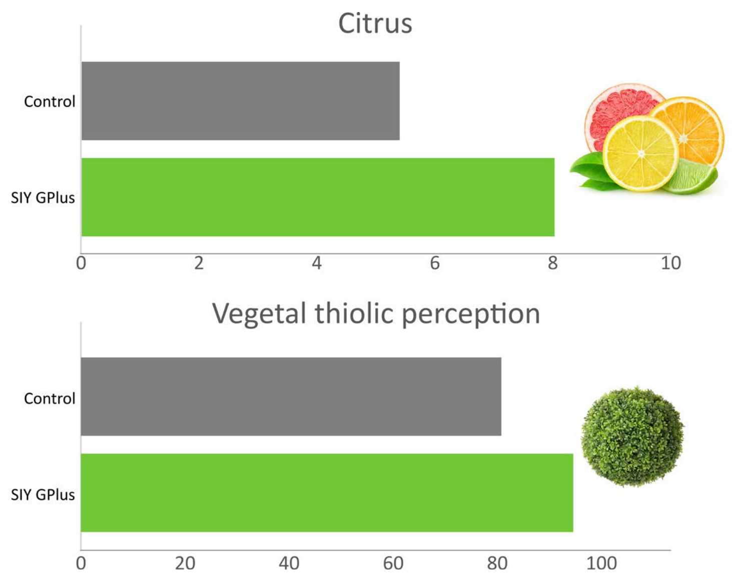

Figure 4. Application of SIY GPlus (marketed as Glutastar) in the free run juice of the press compared to a control with no addition – aroma index based on Odour Value, analysis in bottled wines (Sauvignon Blanc, Loire Valley, France).

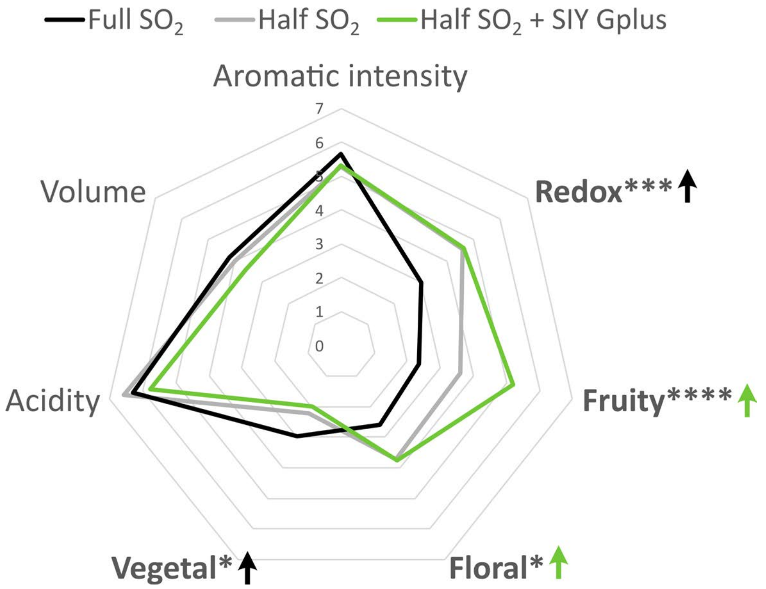

Figure 5. A comparative trial of the application of different levels of SO_2 with and without SIY GPlus (marketed as Glutastar) in the pre-fermentative stage in a Chardonnay (IUVV, Burgundy , France).

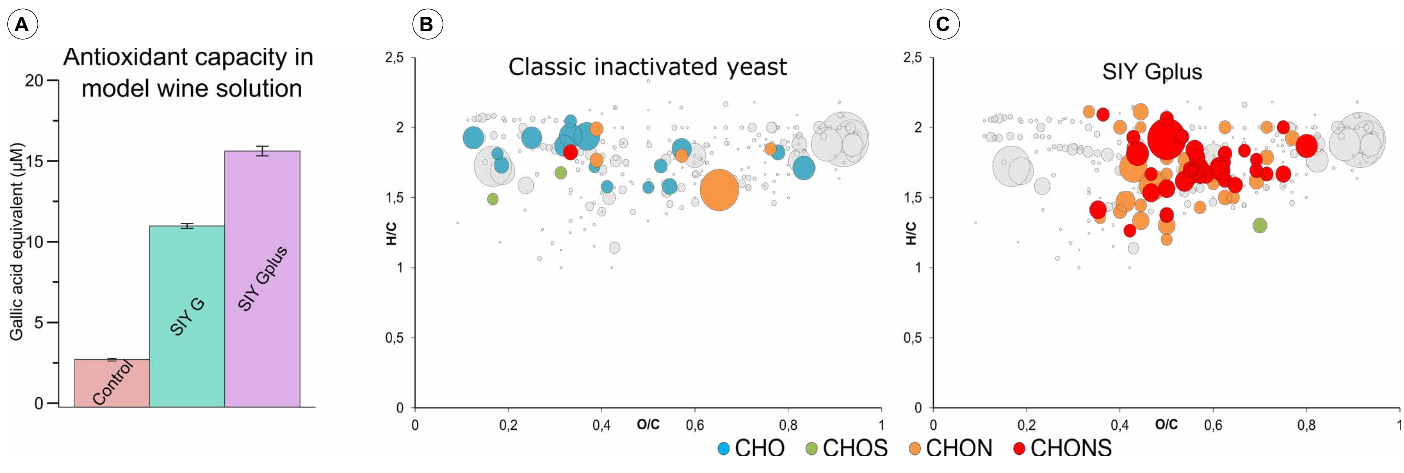

Figure 6. (A) The antioxidant capacity estimated using the DPPH assay for different inactivated yeast from traditional (control) to Glutastar; (B) Mapping of the unique chemical molecules released by a traditional inactivated yeast; and (C) Glutastar in a model solution and detected by high resolution mass spectrometry (Bahut *et al.* 2019, 2020).

antioxidant capacity of inactivated yeast is related to the absolute concentration of GSH and, secondly, the abundance of CHONS (thus peptides containing sulfur) appears to promote better antioxidant capacity.

Thus, it is the combination of a high natural GSH concentration and the unique peptide content in the inactivated yeast that protects wine against oxidation and preserves important varietal aroma compounds, thus confirming those original observations from the early-mid 2000s.

SUMMARY

Even though it is well known that glutathione (GSH) can protect wine against oxidation and aroma compound loss, its use in a pure form is not permitted in winemaking (FSANZ and OIV regulations). SIY GPlus, an inactivated yeast naturally enriched with GSH and other active compounds, offers this protection through both the presence of GSH, a trap for quinone, and with other active compounds (unique peptides, etc.) as traps for free radicals. Finally, implementation of SIY GPlus (Glutastar) at the earliest stages of the white or rosé vinification process ensures an improvement in wine quality by preserving colour and aromas throughout the process and through to bottling. This natural oenological tool can be an alternative to traditional antioxidants, thus enabling a reduction in chemical inputs.

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