OXIDATIVE STABILITY OF WHITE AND ROSÉ WINES

LALLEMAND OENOLOGY

IN THIS ARTICLE WE PRESENT RESEARCH WORK CARRIED OUT IN COLLABORATION WITH THE UNIVERSITY OF BURGUNDY, WHICH HAS HIGHLIGHTED THE IMPACT OF A NEW SPECIFIC INACTIVATED YEAST DEVELOPED FOR THE PROTECTION OF MUSTS AND WINES AGAINST OXIDATION. **BY FLORIAN BAHUT, ANTHONY SILVANO, NATHALIE SIECZKOWSKI, CHRISTIAN COELHO, MARIA NIKOLANTONAKI, PHILIPPE SCHMITT-KOPPLIN & RÉGIS GOUGEON**

INTRODUCTION

White and rosé winemaking requires particular attention to the risks associated with the oxidation phenomena. Colour and aroma are key determinants of wine quality and freshness for these wines and oxidative stability is therefore at the heart of winemakers' concerns, especially given the current trend to limit chemical inputs, particularly sulphites.

In this context, research is leading to a better understanding of wine oxidation mechanisms and the development of new tools aiming at improving the longevity of wines during aging and storage in the cellar and beyond after bottling.

Research work carried out at the IUVV (*Institut Universitaire de la Vigne et du Vin*) have allowed the detailed characterisation of a new inactivated yeast with guaranteed glutathione content that is unique and effective in relation to the oxidative stability of wine.

DEVELOPMENT OF A NEW SPECIFIC INACTIVATED YEAST WITH GUARANTEED GLUTATHIONE CONTENT

Glutathione is a tripeptide that can protect must and wine from oxidation through its ability to react with quinones to form the grape reaction product (GRP), thus preventing browning and loss of aroma in wine. The addition of glutathione, through the use of specific inactivated yeasts naturally rich in glutathione developed since 2003 (Patent No. WO/2005/080543), has proven its worth at the experimental and industrial levels. Inactivated yeasts with guaranteed glutathione content are now included in the OIV Enological Codex.

The uniqueness of inactivated yeasts with guaranteed glutathione content is based on the combination of a specific wine yeast strain and an optimised process ensuring the synthesis of glutathione by the yeast and its accumulation in the reduced form in the intracellular content of the biomass before inactivation. Recently, a considerable improvement in levels of reduced glutathione has been achieved through the use of a yeast strain selected for these unique properties and leading to the development of the inactivated yeast GPlus.

PROPERTIES OF THE NEW SPECIFIC INACTIVATED YEAST DEMONSTRATED BY METABOLOMICS

In traditional chemistry, analytical methods have been developed and optimised to detect and quantify known compounds. New methods have emerged in recent years for analysing all the compounds present in a particular cell or matrix, rather than a single target compound. Metabolomics is one such method, since it allows the instantaneous analysis of all metabolites, i.e. molecules of low mass present in a cell or matrix. This provides insight into the response of a matrix to an environmental modification. Metabolomics offers the possibility of classifying molecules into chemical families (sugars, proteins, lipids, etc.), in particular according to their composition in the elements C, H, O, S and N.

For this metabolomic study, three specific inactivated yeasts (SIYs) were selected:

- Standard inactivated yeast (N) from the "GSHa" strain of *Saccharomyces cerevisiae* and not subject to the process for accumulation of intracellular glutathione, ([reduced glutathione] = 5 mg/g);
- Glutathione-rich inactivated yeast (G) from the "GSHa" strain of *Saccharomyces cerevisiae* and subject to the process for accumulation of intracellular glutathione, ([reduced glutathione] = 18 mg/g);
- Glutathione-rich inactivated yeast (GPlus) from the optimised "GSHb" strain of *Saccharomyces cerevisiae* and subject to the process for accumulation of intracellular glutathione, ([reduced glutathione] = 25 mg/g).

Figure 1A shows the metabolites detected and annotated by mass spectrometry that are common to all three SIYs. Each colour corresponds to a combination of elements (CHO, CHOS, CHONS and CHON) and these metabolites are then separated on a Van Krevelen diagram according to the H/C and O/C ratios. This representation illustrates the chemical diversity of the molecules present in our samples. Comparison of Figures 1B, 1C and 1D allows evaluation of the characteristics, in terms of metabolic diversity, specific to each SIY. It comes out that GPlus has a high density of CHON and CHONS compounds in the region corresponding to peptides. Actually GPlus releases a wide variety of amino-sulphur and amine compounds that correspond to peptides (40 unique peptides compared with seven and two for G and N respectively). Quantitative studies show that the majority of sulphur peptides are released more abundantly by GPlus, which could be of great oenological interest owing to their reducing nature.

BENEFITS FOR IMPROVED OXIDATIVE STABILITY AND QUALITY OF WHITE AND ROSÉ WINES

Antiradical activity

Antiradical activity is a precise and fast measure of the capacity of a matrix to scavenge radicals (oxidising chemical species). A wine capable of resisting these radicals will be able to withstand exposure to oxygen, so the oxidative stability of wine can be described as the capacity of a wine to cope with oxidation reactions.

Figure 2A shows the reactivity of SIYs in model solution against DPPH, a radical made stable using a method optimised for wine. The results are expressed in terms of equivalents of gallic acid, a well-known antioxidant in the scientific literature. These results show the effectiveness of GPlus compared with the other two SIYs (N and G). This can be explained by the quantity of glutathione released by GPlus, but also by the diversity of the reducing compounds.

In a practical application, the use of G and GPlus on a Sauvignon blanc wine at the juice clarification stage allows the effect on this wine to be seen five months after bottling (Figure 2B). The wine with the addition of GPlus shows a stronger antiradical capacity than the control, but also stronger than the wine with addition of G.

Protection of colour

A comparative trial was set up on a pilot scale using a Syrah-Grenache rosé must obtained by direct pressing in Provence in the 2017 vintage. Among different must treatment strategies at the time of clarification, addition (30 g/hL) of GPlus was compared with that of G and an untreated control. The colour was evaluated and the results are projected on the rosé wine colour chart (Figure 3). The wine resulting from early treatment with GPlus shows a colour considered to be of higher quality, with a less orange shade.

Protection of aromatic compounds

During the 2017 and 2018 vintages, numerous trials were carried out. One of them, conducted in the Loire Valley, consisted in evaluating the impact of addition of GPlus to a Sauvignon must subject to two different pre-fermentation processing strategies: classic clarification or cold storage for eight days at 4°C. Two variants were compared for each of these processes: with or without the addition of GPlus at the press outlet.

Thiols, key markers of oxidation, were analysed after bottling (Figure 4) and results demonstrate the protective effect of GPlus regardless of the pre-fermentation process, with varietal thiol levels higher than in the untreated variants. It is also interesting to note the higher level of glutathione in the treated

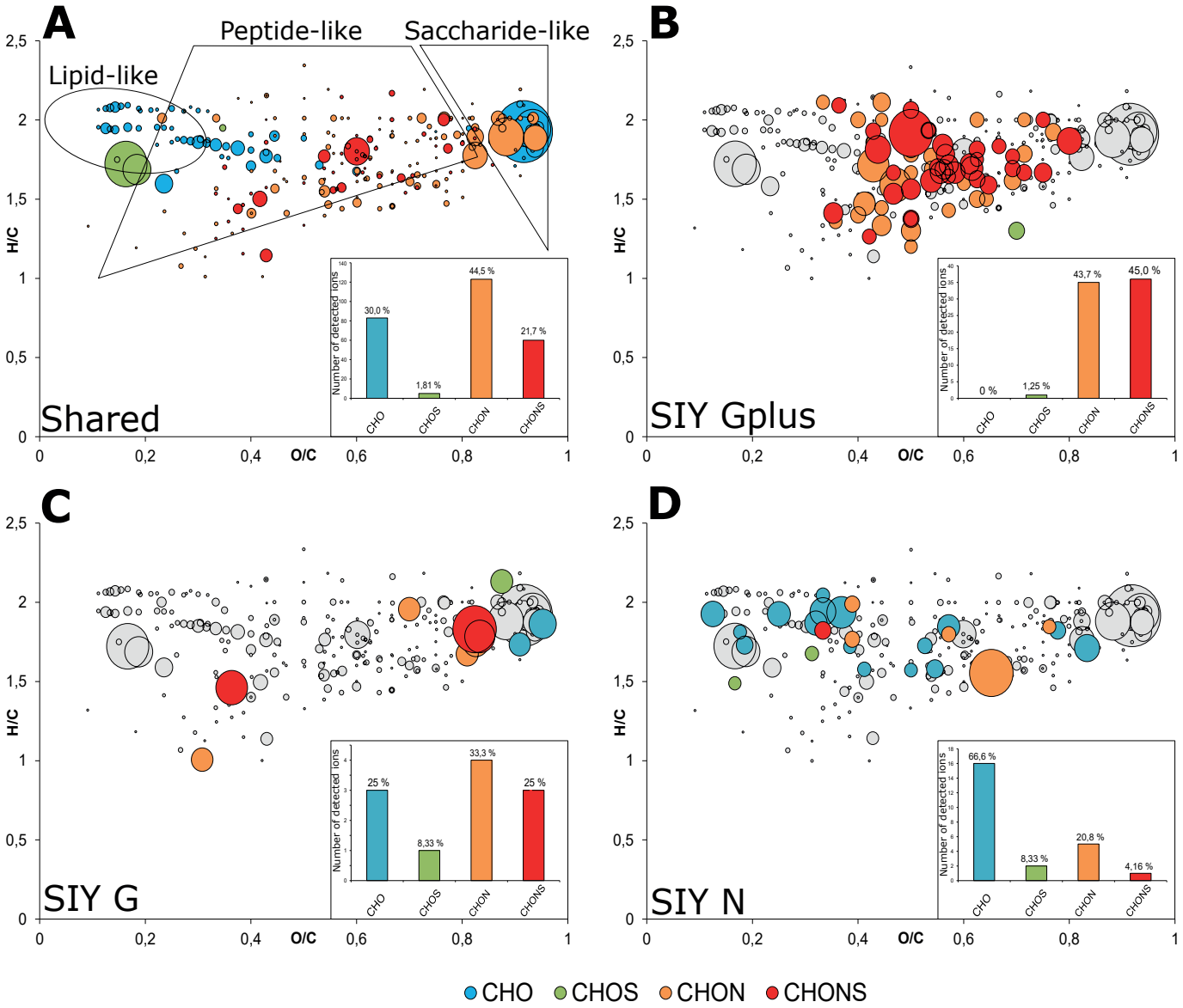

FIGURE 1. Mapping of chemical molecules released in a model wine and detected by very high resolution mass spectrometry in all the specific inactivated yeasts (SIYs) studied (A), only in SIY GPlus (B), only in SIY G (C), or only in SIY N (D).

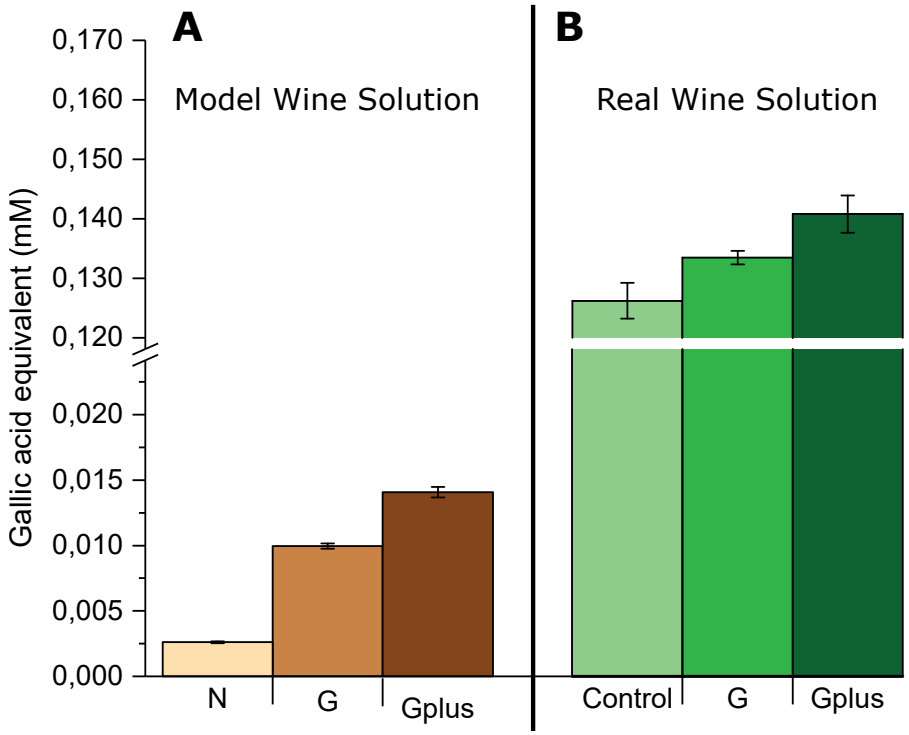

FIGURE 2. Measurement of the antiradical capacity (expressed in gallic acid equivalents) in relation to DPPH, of SIY in model wine solution (A) or Sauvignon blanc wines of the 2018 vintage after five months in bottle (B), with SIY G and SIY GPlus additions of 40 g/hL before clarification; the control corresponds to no addition of SIY. The error bars correspond to six biological replicates (A) and two technical replicates (B).

variants, suggesting improved longevity for these wines.

CONCLUSION

The metabolomics approach used has demonstrated the specific and unique characteristics of GPlus. Beyond the high content of reduced glutathione, the wealth of reducing compounds explains the antiradical activity. Thus the combination of glutathione, a trap for quinone with other active compounds that are traps for free radicals, makes GPlus an innovative tool of choice for the control of wine aging. Finally, implementation at the earliest stages of the white or rosé vinification process ensures better preservation of colour and aromas throughout the process and up to bottling.

SIY GPlus is marketed under the name Glutastar™.

ABSTRACT

This results from the application of an optimised production process to a single strain of *Saccharomyces cerevisiae* yeast to maximise the biosynthesis and accumulation of intracellular glutathione and other compounds of interest.

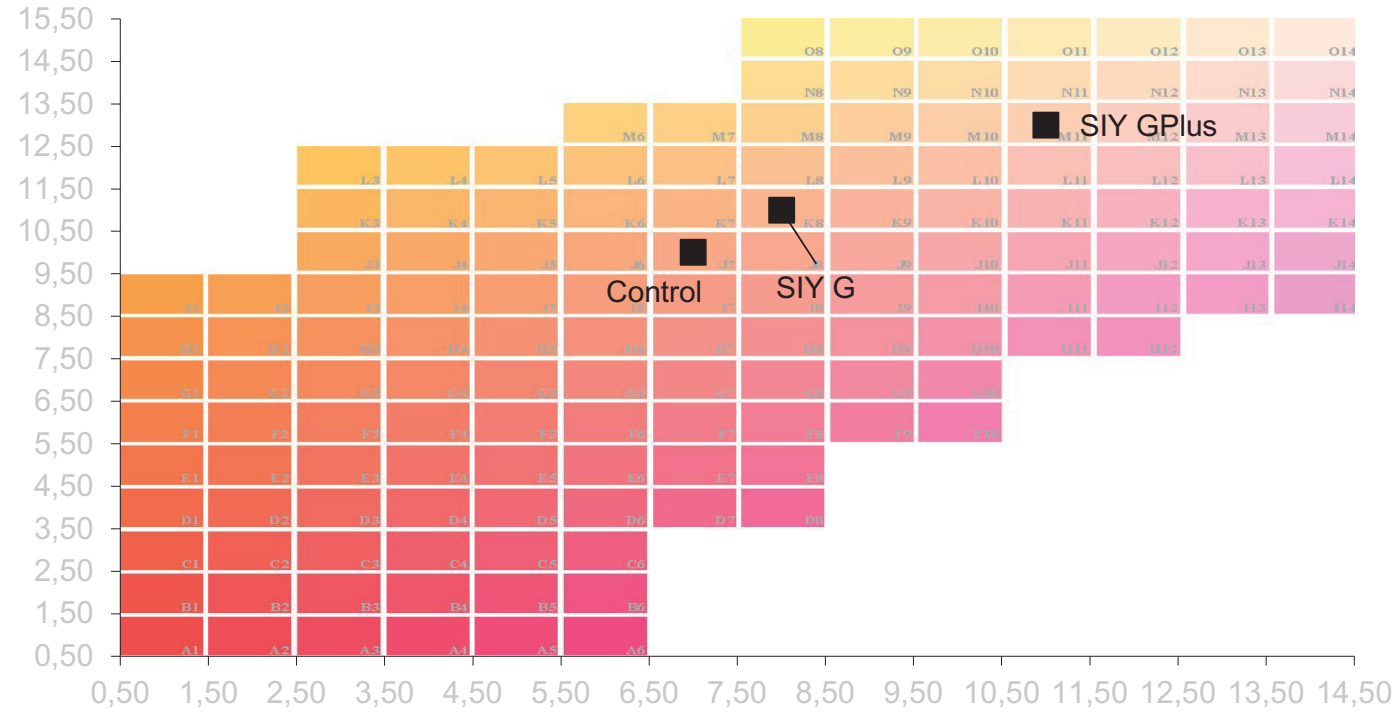

FIGURE 3. Comparative trial of application of SIY at the time of clarification of a Syrah-Grenache rosé must, Provence, 2017: Representation of the wine colour after bottling on the rosé wine colour chart (*Centre du Rosé, IFV, Vidauban*).

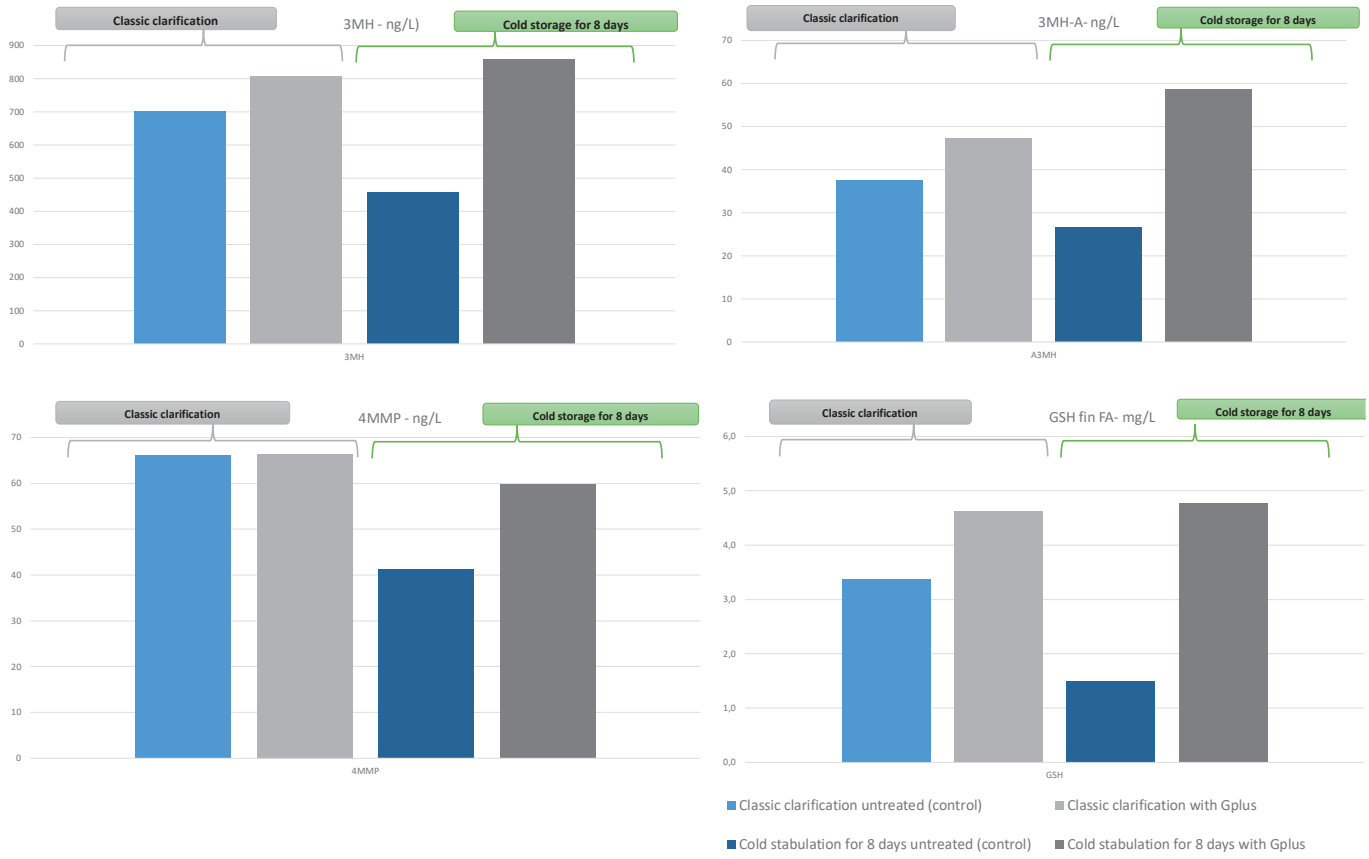

FIGURE 4. Comparative trial, 2018 vintage, Sauvignon blanc, Loire Valley: Analyses of varietal thiols and reduced glutathione in the wines after bottling.

Non-targeted metabolomics characterisation has demonstrated the unique composition of the new inactivated yeast and its impact on wine compared with other inactivated yeasts. In addition to its high content in reduced glutathione, the presence of other reducing peptides further increases the positive impact of this specific inactivated yeast on the oxidative stability of wine.

Numerous application trials have been carried out on white and rosé vinifications during the 2017 and 2018 vintages, and the results show that early treatment before fermentation (after pressing, during clarification or in pre-fermentation cold storage) with the specific inactivated yeast allows for better preservation of aromatic compounds and colour, as well as increased antiradical activity in wines up to bottling.

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