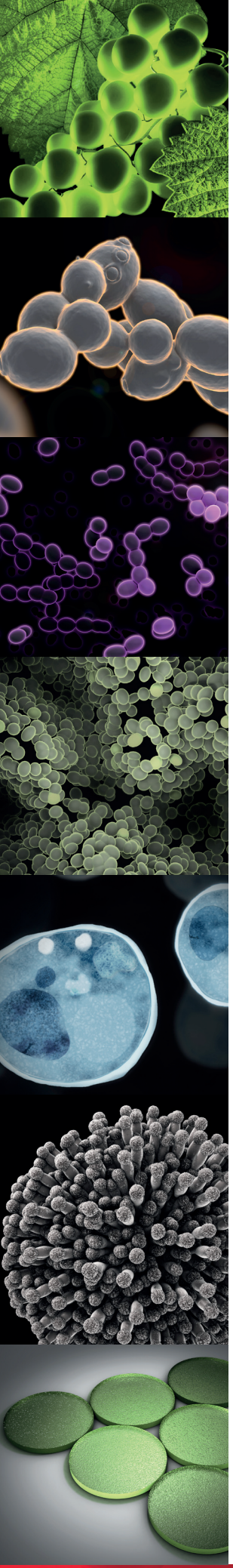

An original and new specific inactivated yeast to improve the oxidative stability of white and rosé wines

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Abstract

In this paper 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.

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

Non-targeted metabolomic characterization has demonstrated the unique composition of the new inactivated yeast and its impact on wine compared with other inactivated yeasts (standard and high glutathione content inactivated yeast). 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, at pilot scale in particular, on white and rosé vinifications during the 2017 and 2018 vintages, to evaluate the impact of this inactivated yeast on wine quality when added before fermentation (after pressing, during clarification or in pre-fermentation cold storage). The results show that early treatment with the specific inactivated yeast allows for better preservation of aromatic compounds and color as well as increased radical-scavenging activity in wines up to bottling.

Introduction

White and rosé winemaking requires particular attention to the risks associated with the oxidation phenomena. Color 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 sulfites.

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

Along with sulfite addition and common fining practices, inactivated yeasts rich in glutathione, which appeared about fifteen years ago, have found their place in winemaking protocols, in order to prevent oxidation in musts and thus preserve the color and aromatic quality of white and rosé wines. They are now included in the OIV Codex with precise specifications giving them the status of inactivated yeasts with guaranteed glutathione content.

Research work carried out at the IUVV (Dijon, Burgundy) has allowed the detailed characterization of a new inactivated yeast with guaranteed glutathione content that is unique and effective in relation to the oxidative stability of wine.

1. 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) (Cheynier et al., 1986) thus preventing browning and loss of aroma in wine. As pure glutathione is not a permitted additive, its indirect addition through the use of specific inactivated yeasts naturally rich in glutathione has been developed since 2003 (Patent No. WO/2005/080543) and has proven its worth at the experimental and industrial levels (Ortiz-Julien and Sieczkowski, 2005, Aguera et al., 2012, Gabrielli et al., 2017).

The uniqueness of inactivated yeasts with guaranteed glutathione content is based on the combination of a specific wine yeast strain and an optimized 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. Inactivated yeast preparations available to winemakers thus have higher or lower glutathione levels depending on the chosen yeast strains and the optimization of the process. Recently, considerable improvement in levels of reduced glutathione has been achieved through the use of a yeast strain selected for its unique properties and leading to the development of the inactivated yeast GPlus.

To characterize its originality and validate its practical interest, work has been carried out in the laboratory, in a model medium and in real musts, and at both pilot and industrial scales.

2. Properties of the new specific inactivated yeast demonstrated by metabolomics

2.1. Application of the metabolomic approach

In traditional chemistry, analytical methods have been developed and optimized to detect and quantify (within a certain concentration range) known compounds or groups of compounds. New methods have emerged in recent years for analyzing 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 (Liu et al., 2018). This provides insight into the response of a matrix (wine, for example) to an environmental modification. Metabolomics offers the possibility of classifying molecules into chemical families (sugars, proteins, lipids...), in particular according to their composition in the elements C, H, O, S and N.

2.2. Metabolomic characteristics of the new specific inactivated yeast

For this metabolomic study, 3 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, having a level of reduced glutathione of approximately 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, and therefore having a level of reduced glutathione greater than 18 mg/g;
- glutathione-rich inactivated yeast (GPlus) from the optimized "GSHb" strain of *Saccharomyces cerevisiae* and subject to the process for accumulation of intracellular glutathione, having a level of reduced glutathione greater than 25 mg/g.

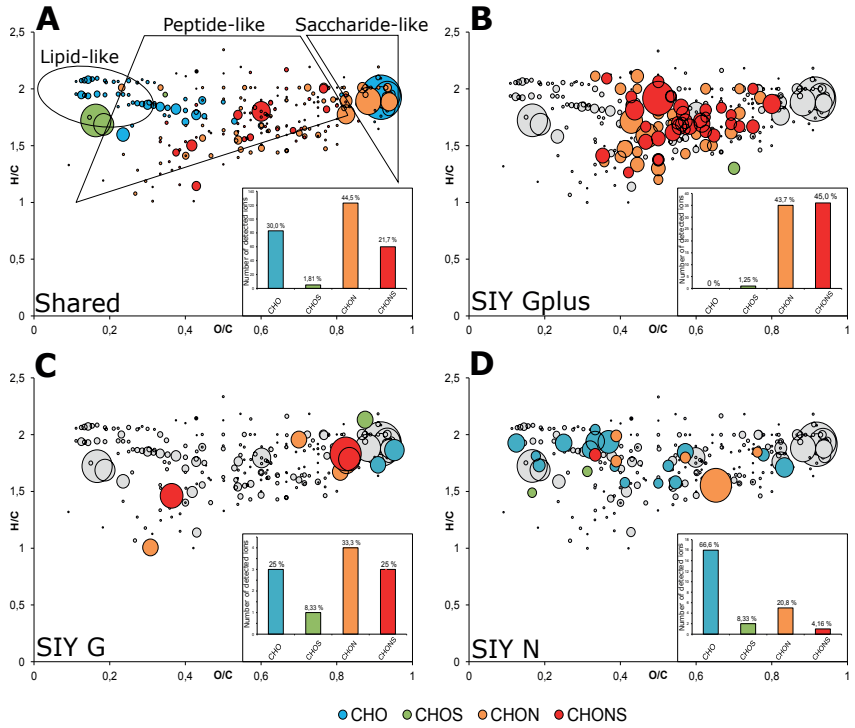

Figure 1: Mapping of chemical molecules released in a model wine and detected by very high resolution mass spectrometry in all the specific inactivated yeasts (SIYs) studied (A) only in SIY GPlus (B) only in SIY G (C) or only in SIY N (D).

Figure 1A shows the metabolites detected and annotated by mass spectrometry that are common to all 3 SIYs. Each color corresponds to a combination of elements (CHO, CHOS, CHONS, 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 the samples. It should be noted that most annotated metabolites are shared between the 3 SIYs (379 compounds), showing that neither the process aiming to optimize glutathione accumulation in the intracellular content nor the yeast strain makes any major change to the primary yeast metabolism during production. Comparison of Figures 1B, 1C and 1D allows evaluation of the characteristics, in terms of metabolic diversity, specific to each SIY. SIY N appears to be richer in lipid compounds that are not found in the other products. In parallel, SIY GPlus has a high density of CHON and CHONS compounds in the region corresponding to peptides. Finally, SIY G, which originates from the same strain as the SIY N and has undergone the same production process as SIY GPlus, has few unique metabolites, since they are found generally in the other 2 SIYs.

SIY GPlus releases a wide variety of amino-sulfur and amine compounds that correspond to peptides (40 unique peptides compared with 7 and 2 for SIY G and SIY N respectively). One of the reasons for this difference is that the process to increase the glutathione accumulation in SIY GPlus also promotes parallel metabolic pathways that result in a higher accumulation of peptides and sulfur peptides for this wine yeast strain specifically. Quantitative studies show that the majority of sulfur peptides (even those shared between the different yeast derivatives) are released more abundantly by SIY GPlus, which could be of great oenological interest owing to their reducing nature (Bahut et al., 2019).

This new specific inactivated yeast GPlus therefore shows innovative metabolic characteristics in comparison with those already available on the market. The large number and diversity of the compounds released could have numerous winemaking implications, on oxidative stability in particular, because of the strong reactivity of the sulfur compounds (notably the thiols) in the interception of the oxidative cascades of the polyphenols.

3. Metabolomic characteristics for oxidative stability of white and rosé wines

3.1 Antiradical activity

Antiradical activity is a precise and fast measure of the capacity of a matrix to scavenge radicals (oxidizing chemical species). In wine, radicals are naturally produced by the reaction of iron and copper (in small quantities) with oxygen. These reactions produce highly reactive species such as hydroxyl radicals and hydroxyethyl radicals (from ethanol). 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.

In the laboratory, it is possible to reproduce these reactions using free radicals which are stable over time and in controlled quantities. These oxidants have a different color once reduced, making it possible to monitor the reduction reaction over time. It is therefore possible to compare the antiradical capacity of different matrices with respect to a specific radical. Figure 2A shows the reactivity of SIY in model solution against DPPH, a radical made stable using a method optimized for wine (Romanet et al., 2019). 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 SIY GPlus compared with the other two SIYs (N and G) since this SIY corresponds to a higher equivalent dose of gallic acid. This difference can be explained by the quantity of glutathione released by GPlus, but also by the diversity of the reducing compounds (notably sulfur peptides) that may be involved in the radical scavenging actions. This first result demonstrates the capacity of this SIY in protection reactions against radical phenomena.

In a practical application, the use of SIY G and SIY GPlus on a Sauvignon Blanc wine (Loire valley, 2018 vintage) at the juice clarification stage allows the effect on this wine to be seen 5 months after bottling (Figure 2B). The wine with the addition of GPlus shows a stronger antiradical capacity than the control wine which received no addition, but also stronger than the wine with addition of SIY G. This shows that the effects of the addition of SIY GPlus during the pre-fermentation phases has a greater beneficial effect on the oxidative stability of wines after bottling, in particular on Sauvignon Blanc, even in the case of the use of the reference inactivated yeast with a guaranteed glutathione content.

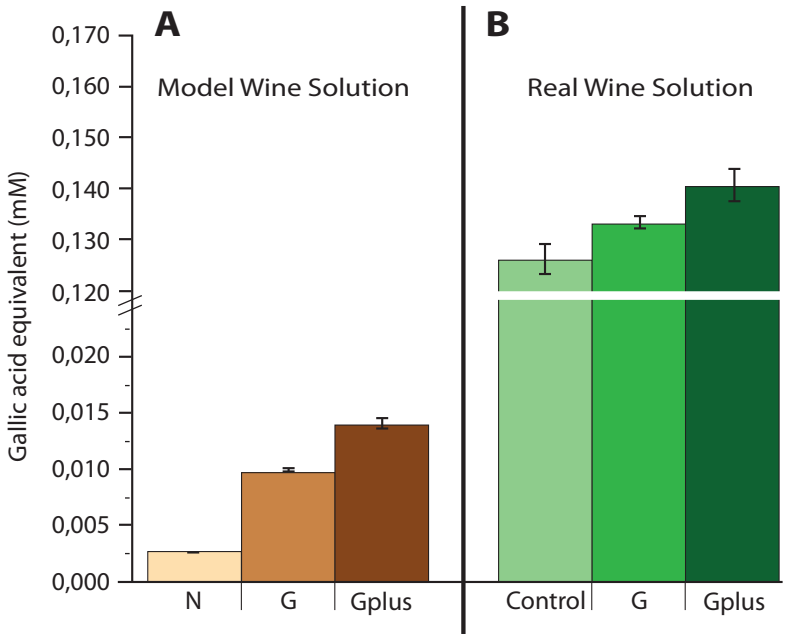

Figure 2: Measurement of the antiradical capacity (expressed in gallic acid equivalents) in relation to DPPH, of SIY in model wine solution (A) or Sauvignon Blanc wines of the 2018 vintage after five months in bottle (B), with SIY G and SIY GPlus additions of 40 g/hL before clarification; the control corresponds to no addition of SIY. The error bars correspond to 6 biological replicates (A) and 2 technical replicates (B).

3.2 Protection of color

A comparative trial was set up at the experimental winery of the Pôle National Rosé/ Centre du Rosé, Vidauban (Provence, France) on a pilot scale using a Syrah-Grenache rosé must obtained by direct pressing in the 2018 vintage. Among different must treatment strategies at the time of clarification, addition (30 g/hL) of SIY GPlus was compared with that of SIY G and an untreated control. Wines made under standard conditions and after the different treatments were monitored until bottling. In particular, the color was evaluated and the results of the analyses are projected on the rosé wine color chart (Centre du Rosé, IFV, France, Figure 3). The wine resulting from early treatment with SIY GPlus shows a color considered to be of higher quality, with a lower orange shade.

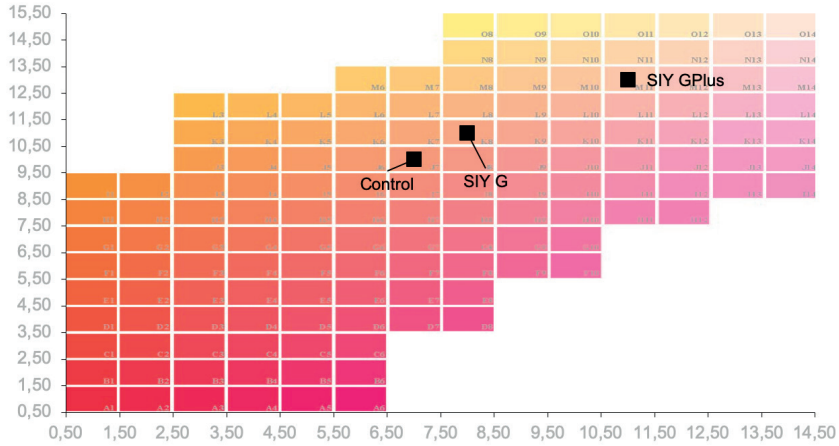

Figure 3: Comparative trial of application of SIY at the time of clarification of a Syrah-Grenache rosé must, Provence, 2018: Representation of the wine color after bottling on the rosé wine color chart (Centre du Rosé, IFV, Vidauban. https://centredurose.fr/nuanciers-vins-roses/)

3.3 Protection of aromatic compounds

During the 2017 and 2018 vintages, numerous trials were carried out in white winemaking, notably on Sauvignon Blanc, a grape variety that is ideal to study the oxidative stability of aromas because of its thiols, key markers of oxidation.

One of these trials, conducted in the Loire Valley, evaluated the impact of addition of SIY GPlus to a Sauvignon Blanc must subject to two different pre-fermentation processing strategies: classic clarification or cold storage for 8 days at 4°C. Two variants were compared for each of these processes: with or without the addition of SIY GPlus at the press outlet.

The resulting wines were the subject of numerous physicochemical and sensory analyses. The thiols were analyzed after bottling and the results presented in Figure 4 demonstrate the protective effect of SIY 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 variants, suggesting improved longevity for these wines.

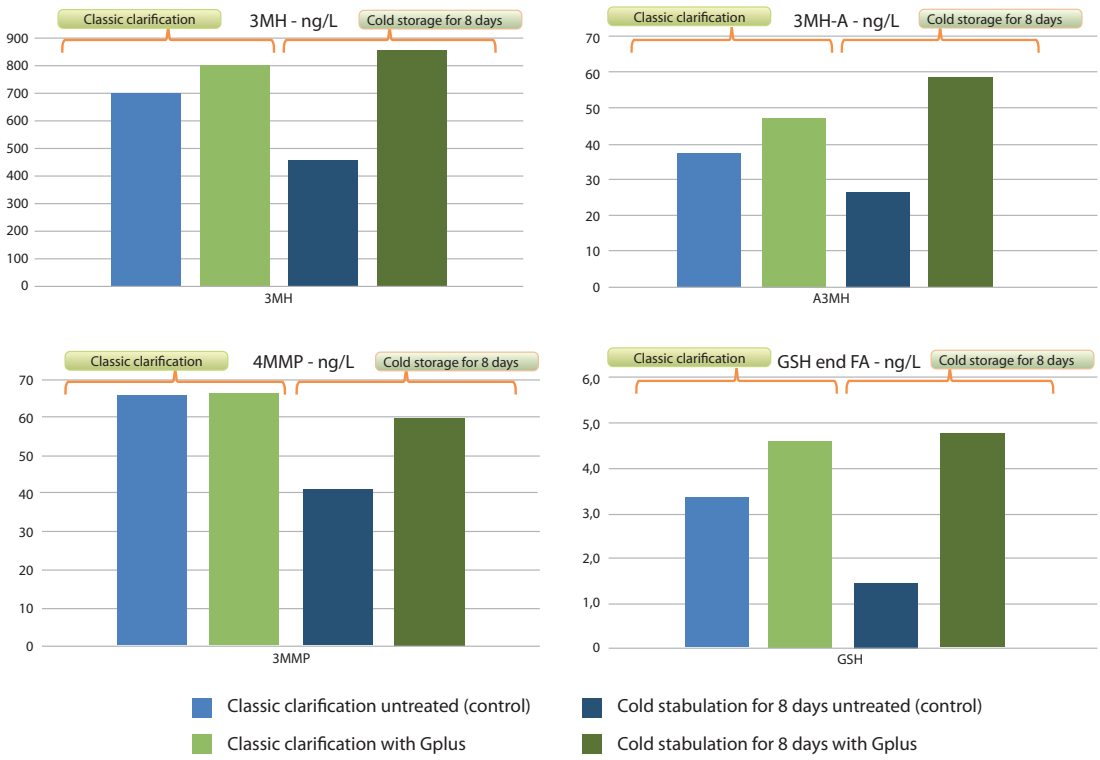

Figure 4: Comparative trial, 2018 vintage, Sauvignon Blanc, Loire Valley: analyses of varietal thiols and reduced glutathione in the wines after bottling

Conclusion

The metabolomic approach used has demonstrated the specific and unique characteristics of SIY GPlus that explain its impact on the oxidative stability of wines. Beyond the high content of reduced glutathione, the wealth of reducing compounds explains the antiradical activity of this tool. This property accentuates its protective effect in relation to multiple oxidative mechanisms. Thus the combination of glutathione, a trap for quinone with other active compounds that are traps for free radicals, makes it 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 color and aromas throughout the process and up to bottling.

SIY GPlus is marketed under the name Glutastar™.

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