LEVEL² INITIA™ –

YEAST FOR BIOPROTECTION OF WHITE AND ROSÉ JUICE IN THE PRE-FERMENTATION PHASE

SHUTTERSTOCK

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INTRODUCTION

The pre-fermentation phase during the vinification of white and rosé wines significantly increases the quality of the wines, but also carries the risk of oxidation and the development of spoilage microorganisms. These risks may result in loss of quality.

In recent years, changes in winemaking practices, regulations and consumer demand have resulted in a significant decrease in the use of sulphites.

Increasingly, winemakers are considering bioprotection of musts using non-*Saccharomyces* yeast. Each species of these yeast has its own specific contribution and differences between strains can also be observed within the same species. Bioprotection with non-fermenting non-*Saccharomyces* yeast is an innovative alternative solution to protect wines from oxidation and spoilage microorganisms.

A new selection of *Metschnikowia pulcherrima* was isolated by IFV Beaune (France) from an original collection of more than 500 strains, for its strong capacity to rapidly consume dissolved oxygen. This new selected yeast is Level² Initia™.

BIOPROTECTION OF WHITE AND ROSÉ JUICE IN THE PRE-FERMENTATION PHASE

To ensure the smooth progress of white and rosé pre-fermentation phases, a yeast used for bioprotection at this stage must have certain oenological properties. Very low fermentation activity and a good multiplication capacity at low temperature are essential prerequisites, along with protection against oxidation.

BIOPROTECTION AGAINST OXIDATION

A trial was carried out in 2020 with the Università degli Studi di Udine (Italy) on 50 kg of Sauvignon blanc grapes divided into three homogeneous batches. One batch was inoculated with Level² Initia™ at 20 g/hL due to the high pH (3.9) of the juice and a strong presence of indigenous microflora (the usual recommended dose is 10 g/hL), before pre-fermentation maceration for 24 hours at 8°C. The other two batches were directly pressed and with or without addition of SO_2 at 4 g/hL. After the pre-fermentation phase and subsequent clarification, the three batches were inoculated with the same *Saccharomyces cerevisiae*. The dissolved oxygen content was measured in the free-run juice from the press. The batch inoculated with Level² Initia™ and the batch with added sulphite both showed the same ability to maintain a minimal level of dissolved oxygen (Figure 1). The protection from oxidation was visible to the naked eye in the colour of the juice (Figure 2).

Another trial was carried out in 2020 at a wine estate in Valencia (Spain) on 100 hL of Sauvignon blanc. Inoculation with Level² Initia™ was compared with another non-*Saccharomyces* yeast, also selected

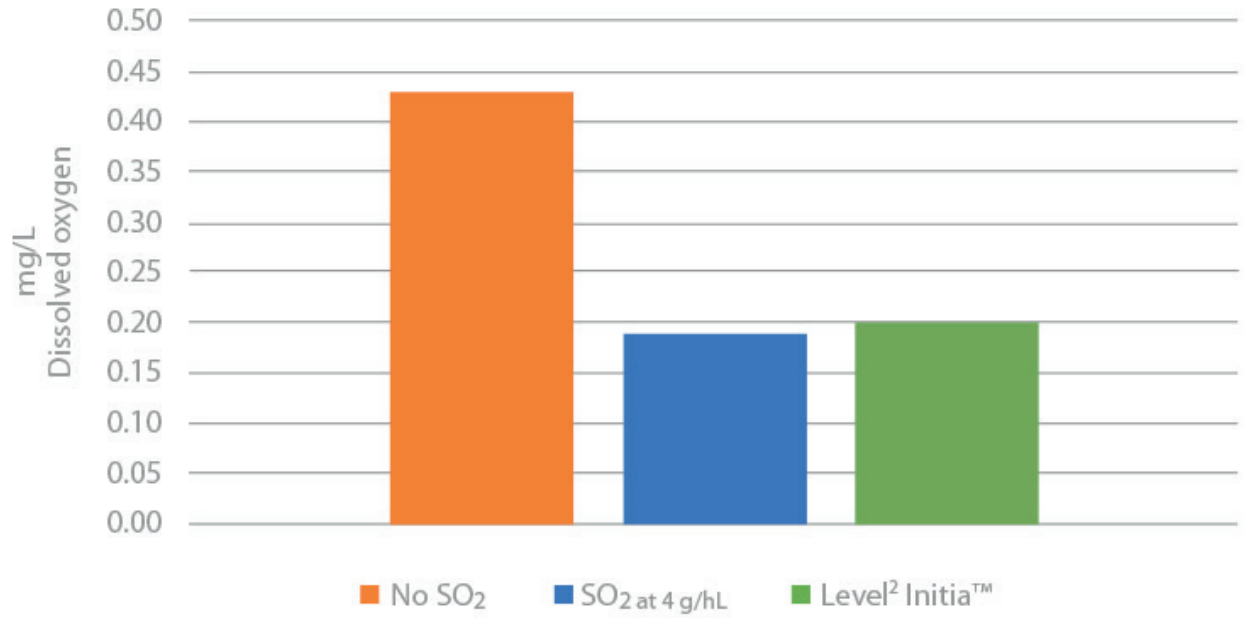

FIGURE 1. Measurement of dissolved oxygen in the press juice with SO_2 or Level² Initia™.

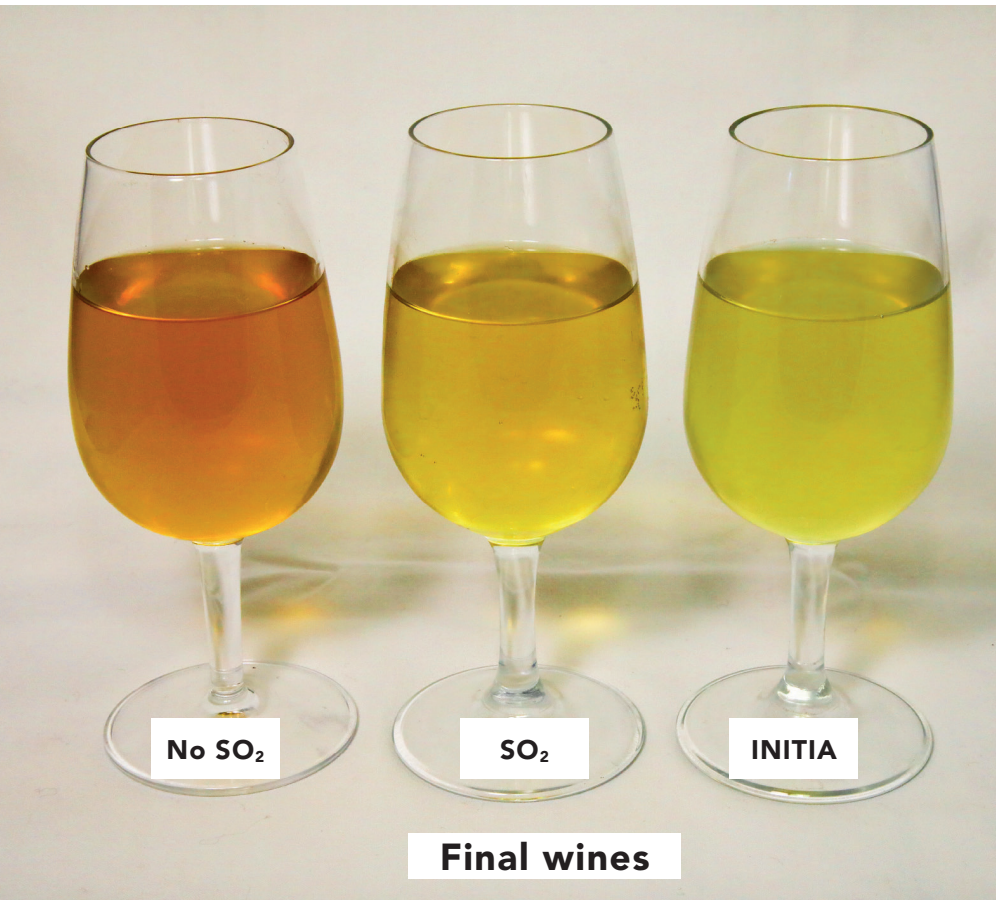

FIGURE 2. Colour of a Sauvignon blanc juice after pre-fermentation maceration for 24 hours at 8°C (Università degli Studi di Udine, Italy, 2020).

showed the highest membrane concentration of poly-unsaturated fatty acids, such as linoleic and linolenic acids, while only trace quantities of these lipid compounds were found in other yeasts. The synthesis requires substantial consumption of oxygen. Our characterisation of different *Metschnikowia pulcherrima* strains demonstrates the variability within the different strains. Level² Initia™ was selected for its strongest capacity to consume oxygen.

In addition to this phenotype, Level² Initia™ revealed a tendency to decrease the copper content of juice. A laboratory-scale trial was carried out on a Sauvignon blanc must to which 6.3 mg/L of initial copper had been added. Inoculation with Level² Initia™ at 10 g/hL reduced the copper content by 41% in 24 hours and by 57% in 48 hours. While the exact mechanisms remain unclear, there is again intra-species variability. As shown in Figure 4, Level² Initia™ differs from other *Metschnikowia pulcherrima* strains in its ability to decrease copper concentrations in juice.

Oxygen plays a key role, both for enzymatic oxidation reactions (under the action of polyphenol oxidase) and for chemical oxidation. In addition, copper is a catalyst for these reactions. By contributing to the reduction in both dissolved oxygen and copper content, Level² Initia™ makes a dual contribution to limiting these oxidation phenomena.

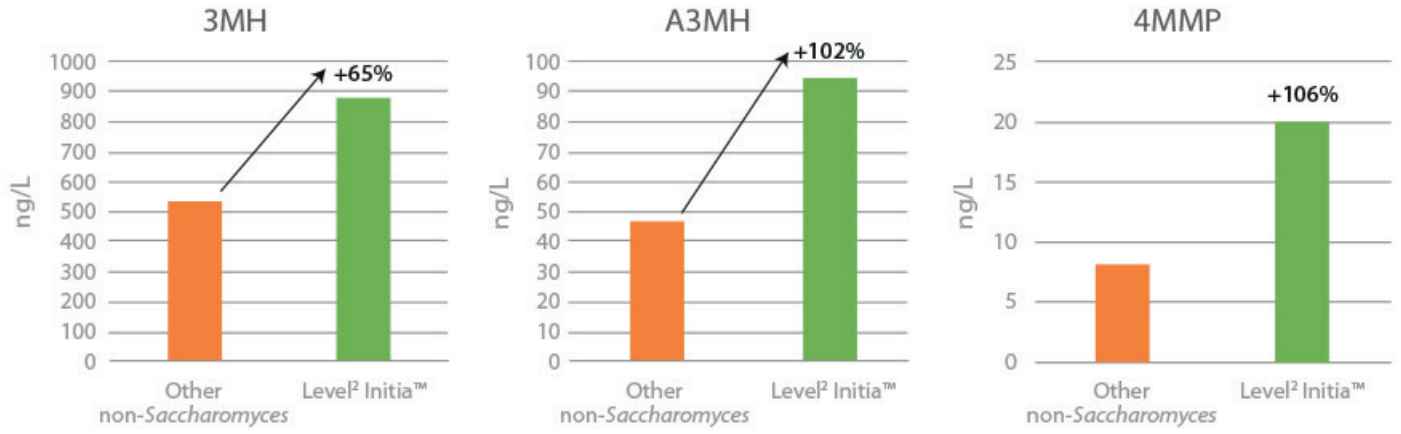

FIGURE 3. Analysis of 3MH, 3MHA and 4MMP thiols at bottling, Sauvignon blanc 2020 (Valencia, Spain).

for bioprotection applications. Each yeast was added at a dose of 10 g/hL before cold juice stabulation [pre-fermentative cold treatment] for five days at 4°C. The batches, subsequently inoculated with the same *Saccharomyces cerevisiae*, exhibited similar fermentation kinetics. Analysis of thiols at bottling showed enhanced preservation of 3MH, 3MHA and 4MMP in the batch inoculated with Level² Initia™ (Figure 3).

The Level² Initia™ yeast thus allowed biocontrol of oxidation under these real winemaking conditions.

MECHANISM OF ACTION

Metschnikowia pulcherrima yeasts do not have the ability to efficiently absorb lipids (poly-unsaturated fatty acids and phytosterols) from grapes, and must therefore synthesize these lipid compounds. Incorporation of lipids into the membrane is essential for their survival. A recent study compared the membrane compositions of *Saccharomyces cerevisiae* and non-*Saccharomyces* yeasts at the end of the alcoholic fermentation. *Metschnikowia pulcherrima*

MICROBIOLOGICAL BIOPROTECTION

The oenological interest of Level² Initia™ also lies in its microbiological bioprotection. Microbiological monitoring of *Hanseniaspora uvarum* and *Brettanomyces* populations was performed in a Chardonnay (Sicarex Beaujolais).

There was a significant total yeast population in the juice, of which more than one-third was *Hanseniaspora uvarum*. At the end of cold stabulation, 50% of

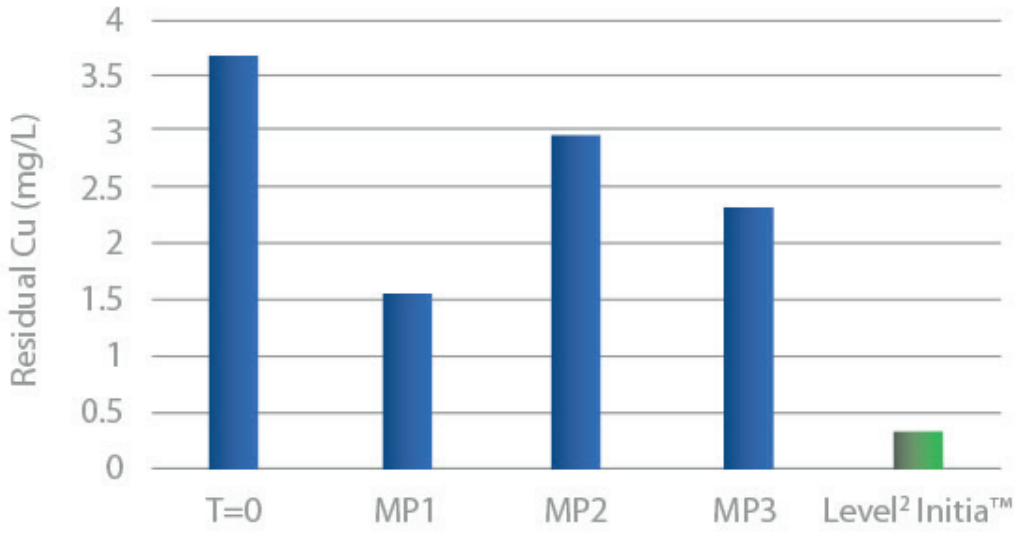

FIGURE 4. Residual copper in a Sauvignon blanc juice with different strains of *Metschnikowia pulcherrima* including Level² Initia™.

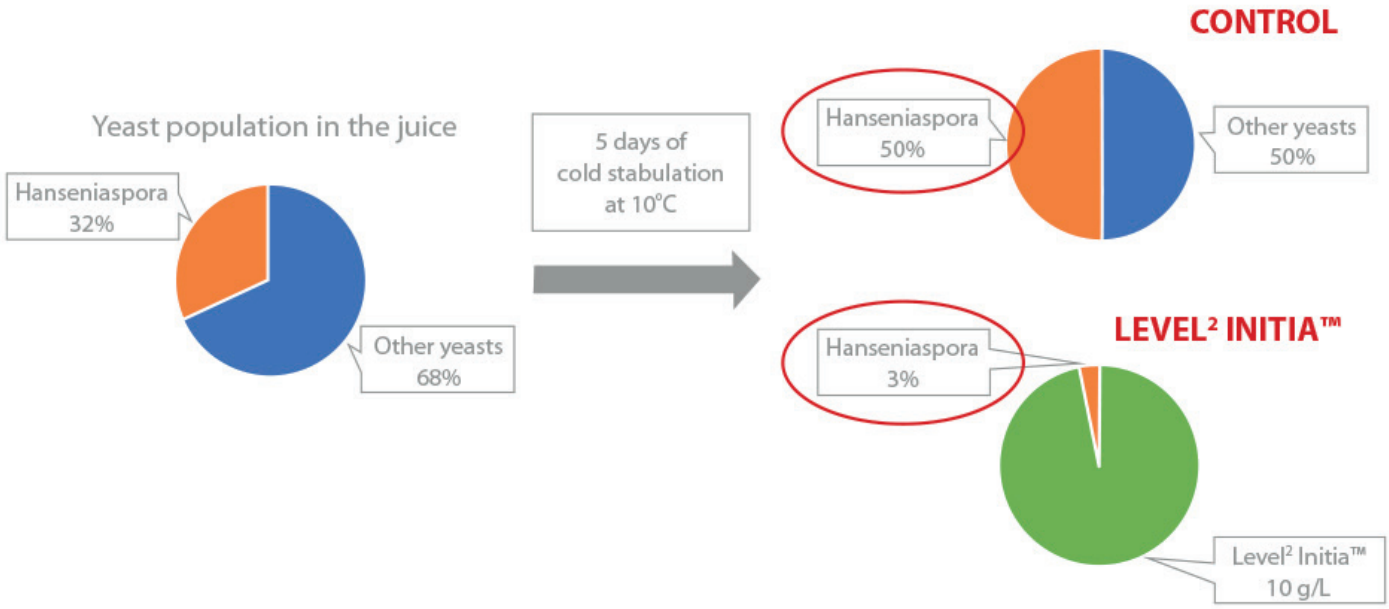

FIGURE 5. Microbiological bioprotection by Level² Initia™ (Chardonnay no SO_2, Sicarex Beaujolais, France).

the yeasts in the non-bio protected batch were *Hanseniaspora* compared with 3% for the batch with Level² Initia™ (Figure 5). The other yeasts present in the non-bio protected batch possibly included *Saccharomyces* and non-*Saccharomyces* species originating from the indigenous flora.

This trial, along with several others in wineries, thus confirms that Level² Initia™ can act on spoilage microflora and/or undesirable microflora. *Metschnikowia* occupies the microbiological space in the juice and prevents excessive growth of spoilage microorganisms, reducing the risk of undesirable compounds developing in the wine.

CONCLUSION

In a context of global warming and rising pH levels, the risks associated with unwanted microflora are increasing, especially as SO_2 is no longer always sufficiently active and its use is increasingly avoided by winemakers and not wanted by consumers. In addition, white and rosé juice are particularly sensitive to the oxidation phenomenon, which appears as soon as the grapes are harvested. The new selected yeast Level² Initia™ performs a full bioprotection role by limiting oxidative, enzymatic and microbiological contamination phenomena and is therefore shown to be a valuable tool for limiting sulphite additions during the pre-fermentation phases in white and rosé vinification, while preserving the organoleptic potential of the wines.

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Note: Article previously published in *Revue des Oenologues.*

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