**The potentiality of non-*Saccharomyces* yeast derivatives**

**as enological bio-adjuvants**

Valentina Civa (valentina.civa@unifi.it)

Dpt. of Agriculture, Food, Environment and Forestry (DAGRI) - University of Florence, Florence, Italy

Tutor: Prof. Paola Domizio

To date, the yeast derivatives allowed in winemaking are only those obtained from strains belonging to the genus *Saccharomyces* and there are many scientific works that have highlighted their impact on the qualitative characteristics of wine. Conversely, derivatives obtained from non-*Saccharomyces* yeasts have not yet been accepted in winemaking, despite their use as mixed starters for alcoholic fermentation have already been accepted since several years. The advantages deriving from their use in winemaking have been associated not only with their specific metabolism during alcoholic fermentation, but also with the composition of their cell wall, with particular regard to the polysaccharide fraction. Therefore, after having produced some inactivated non-*Saccharomyces* yeast derivatives on a laboratory scale, we proceeded with their evaluation, initially on a model solution and subsequently on a Trebbiano white wine. In particular, their impact on protein stability, colour and aromatic profile was evaluated.

**Derivati di lieviti non-*Saccharomyces* come potenziali bio-coadiuvanti enologici**

Ad oggi i derivati di lievito ammessi in vinificazione sono solo quelli ottenuti a partire da ceppi appartenenti al genere *Saccharomyces* e molteplici sono i lavori scientifici che hanno evidenziato il loro impatto sulle caratteristiche qualitative del vino. Al contrario, i derivati ottenuti da lieviti non-*Saccharomyces* non sono ancora stati ammessi in vinificazione, nonostante il loro utilizzo come starter misti per la fermentazione alcolica sia stato già ammesso da diversi anni. I vantaggi derivanti dal loro utilizzo in vinificazione sono stati associati non solo al loro specifico metabolismo durante la fermentazione alcolica, ma anche alla composizione della loro parete cellulare, con particolare riguardo alla frazione polisaccaridica. Pertanto, dopo aver prodotto su scala di laboratorio alcuni derivati inattivati di lieviti non-*Saccharomyces*, si è proceduto con la loro valutazione, inizialmente su soluzione modello e successivamente su un vino bianco Trebbiano. In particolare è stato valutato il loro impatto sulla stabilità proteica, sul colore e sul profilo aromatico.

**Key words**: non-*Saccharomyces yeast*, yeast derivatives, colloidal stability, antioxidant activity, aromatic profile

# **1. Introduction**

In accordance with the PhD thesis project, this poster reports the main results of the first activities concerning:

(A1) characterization of IDYs in wine like solution

(A2) evaluation of IDYs impact on a Trebbiano white wine.

# **2. Materials and Methods**

*Saccharomycodes ludwigii* (SL) and *Starmerella bacillaris* (SB) represent two of the eight non-*Saccharomyces* strains used as inactivated dry yeasts (IDYs) in this PhD project, and selected for the present report. A commercial strain of *Saccharomyces cerevisiae* (SC) was used as reference strain for *Saccharomyces* and for comparison determination.

For the first activity (A1), IDYs were added onto a wine like solution (ethanol 12% v/v, tartaric acid 4.5 g/L, pH 3.2), and kept in contact for 48 h. After that, polysaccharides quantification (Romani et al, 2020) and evaluation of their molecular weight profiles (Fanzone et al, 2012), were performed. Quantification of GSH (Tirelli et al, 2010) was also determined.

For the second activity (A2), the IDYs were added onto a Trebbiano wine and left in contact for 15 days. After that, the following analyses were performed: quantification of total polysaccharides; evaluation of wine protein stability (by the heat test: 80°C for 2h, 4°C for 16h, RT for 2h) and of colour indexes (by CIELab); quantification of the aroma compounds (by SPE-GC/MS).

Figure 1 Concentration of total polysaccharides(mg/g) released in a wine like solution by the IDY (SC, SL, SB LSD -Least Significant Difference, Anova Fisher’test Different Letters Indicate Statistical Significances,)

**3. Results and Discussion**

**3.1 Characterization of IDYs in wine like solution**

In comparison with IDY- SC, IDY-SL and IDY-SB, showed a higher capacity to release polysaccharides in the media (figure 1). These results are likely due to their specific cell wall composition. Moreover, both IDY-SL and IDY-SB showed a higher concentration of polysaccharides with molecular weight > 250 kDa, as compared to those of IDY-SC. Considering that polysaccharides with different molecular weight have been shown to have different effect on the wine colloidal stability, further researches are needed to evaluate their relevant impact.

Regarding GSH, each IDYs was able to release it in the solution in different quantities. This is an important feature, permitting to use IDYs also to possibly reduce the addition of SO₂ during the winemaking process.

## **3.2 Impact of IDYs in a Trebbiano white wine**

All the wine samples treated with IDY showed amounts of total polysaccharides in agreement with those found in the wine like solutions. Moreover, each IDY was able to improve the protein stability of the corresponding treated wine. However, contrary to what was expected, the decrease of ΔNTU, did not not result correlated with the amount of polysaccharides. Indeed, the wine treated with IDY-SB showed the lowest values of protein stability, although it contained the highest amount of polysaccharides in the respective wine.

In comparison with the control, all treated wines presented lower absorption in the yellow wavelength, were brighter, and were less red (a\* negative values *versus* the control) and less yellow (b\* lower values *versus* the control), (Table 1). These results might be due to an antioxidant activity of the IDYs, or to an adsorption of the phenolic compounds by the inactivated yeasts cell. In this regard, we are conducting specific investigations in order to understand better their mechanism of reaction.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sample** | **A420** | **L\*** | **a\*** | **b\*** |
| CT  | 0.099±0.001 | 97.4±0.078 | 0.29±0.040 | 6.63±0.018 |
| SC  | 0.084±0.005 | 97.9±0.362 | -0.30±0.036 | 5.55±0.098 |
| SL  | 0.084±0.002 | 98.0±0.100 | -0.23±0.028 | 5.58±0.051 |
| SB | 0.076±0.001 | 98.7±0.044 | -0.35±0.020 | 5.35±0.022 |

***Table 1*** *Absorption at 420 nm and CIELab coordinates(L\*,a\*,b\*)* *of Trebbiano white wines (mean value ± standard deviation) evaluated 15 days after the addition of the inactivated dry yeast (SC, SL, SB). CT: Trebbiano wine without IDY treatment.*

Regarding wine aromatic profile, each IDY have shown a different impact (Table 2); in particular, IDY-SC determined a lower decrease of higher alcohols, in comparison with both IDY-SL and IDY-SB. Interestingly, the yeast derivative IDY-SL determined no decrease of ethyl and acetates esters, compared with the control as well as with the other two yeast derivatives (IDY-SC and IDY-SB). Further investigations are currently underway to understand whether the impact on aromatic compounds is due to their interaction with the polysaccharides released in the wine or with those still present in the cell wall. On the other hand, some authors have found similar results and they hypothesized a hydrophobic interaction between specific aromatic compounds and the polysaccharides (Chalier et al, 2007).

***Table 2*** *Main volatile compounds of Trebbiano white wines (mean value ± standard deviation) evaluated 15 days after the addition of the inactivated dry yeast (SC, SL, SB). CT: Trebbiano wine without IDY treatment.*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Volatile compounds** | **CT** | **SC** | **SL** | **SB** |
| Higher Alcohols (mg/L) | 167.60±29.66 | 163.64±7.39 | 154.65±10.98 | 151.61±7.17 |
| Carboxylic acid esters (mg/L) | 50.25±7.62 | 36.23±4.63 | 44.56±1.25 | 38.61±6.40 |
| Fatty acids (mg/L) | 27.06±3.28 | 28.48±2.27 | 25.29±1.19 | 23.68±1.43 |
| Ethyl esters (μg/L) | 2861.40±544.59 | 2365.05±277.06 | 2870.31±145.75 | 2525.60±425.7 |
| Acetates ester (μg/L) | 3537.53±284.48 | 3293.87±212.16 | 3548.93±219.16 | 3122.97±504.66 |
| Terpens (μg/L) | 88.88±16.27 | 81.44±13.13 | 74.18±9.12 | 64.32±6.72 |

# **4. References**

Chalier, P., Angot, B., Delteil, D., Doco, T., & Gunata, Z. (2007). Interactions between aroma compounds and whole mannoprotein isolated from Saccharomyces cerevisiae strains. *Food chemistry*, *100*(1), 22-30.

Fanzone, M., Peña-Neira, A., Gil, M., Jofré, V., Assof, M., & Zamora, F. (2012). Impact of phenolic and polysaccharidic composition on commercial value of Argentinean Malbec and Cabernet Sauvignon wines. *Food Research International*, *45*(1), 402-414.Cheryan M (1998) *Ultrafiltration and Microfiltration Handbook*, Lancaster (USA): Technomic Publ. Co.

Romani, C., Lencioni, L., Biondi Bartolini, A., Ciani, M., Mannazzu, I., & Domizio, P. (2020). Pilot scale fermentations of Sangiovese: an overview on the impact of Saccharomyces and Non-Saccharomyces Wine Yeasts. *Fermentation*, *6*(3), 63.

Tirelli, A., Fracassetti, D., & De Noni, I. (2010). Determination of reduced cysteine in oenological cell wall fractions of Saccharomyces cerevisiae. *Journal of agricultural and food chemistry*, *58*(8), 4565-4570.