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# The use of *Torulaspora delbrueckii* in carbonic maceration wines modulates their organoleptic properties and favours malolactic fermentation

**Candela Ruiz-de-Villa[1], Jordi Gombau[2], Montse Poblet[1], Albert Bordons[3], Joan Miquel Canals[2], Fernando Zamora[2], Cristina Reguant[3], Nicolas Rozès[1*]**

[1] Grup de Biotecnologia Microbiana dels Aliments, Rovira i Virgili University, Faculty of Oenology, Biochemistry and Biotechnology Department, Marcel·lí Domingo 1, 43007 Tarragona, Catalonia, Spain
[2] Grup de Tecnologia Enològica, Departament de Bioquímica i Biotecnologia, Facultat d'Enologia, Universitat Rovira i Virgili, c/ Marcel·lí Domingo s/n, 43007 Tarragona, Catalonia, Spain
[3] Grup de Biotecnologia Enològica, Rovira i Virgili University, Faculty of Oenology, Biochemistry and Biotechnology Department, Marcel·lí Domingo 1, 43007 Tarragona, Catalonia, Spain

Recently, there has been growing interest in the study of non-*Saccharomyces* yeasts due to both the benefits they bring in the wine production process and their organoleptic properties. The use of the species *Torulaspora delbrueckii* in alcoholic fermentation (AF) together with *Saccharomyces cerevisiae* (Sc) has also been observed to have a positive effect on the performance of malolactic fermentation (MLF)[1]. This effect has been little studied in special winemaking, such as in carbonic maceration (CM), which consists of developing the FAL within the unpressed grape berries in an anaerobic atmosphere, thus producing very aromatic wines[2]. The objective of this study is to investigate the oenological implications of inoculation of *T. delbrueckii* during the first stage of vinification by CM, in order to improve the fermentation process and the organoleptic characteristics of these wines.

## Materials and methods

Fermentations were carried out in small volumes in 15 L plastic containers using the Red Garnacha grape variety from the *Mas dels Frares* winery (Rovira i Virgili University, DO Tarragona). To carry out carbonic maceration (CM), the grapes were destemmed by hand, and whole uncrushed grape berries were placed in the containers, introducing $CO_2$ to achieve an anaerobiosis environment (Figure 1). The conditions were as follows: two sequential conditions in which two strains of *T. delbrueckii* were inoculated with Viniflora Prelude (Chr. Hansen Holding AS, Hoersholm, Denmark) and Viniferm NS Td (Agrovin, Alcázar de San Juan, Spain), and the control without inoculation. After 5 days of CM at 20°C, the grapes were pressed and the must fractions mixed. The fermented must from each condition was racked and inoculated with *S. cerevisiae* CLOS (Lallemand Inc., Montreal, QC, Canada) to complete the alcoholic fermentation. Finally, three malolactic fermentations (MLF) were carried out using two different strains of *Oenococcus oeni* (Lalvin VP41 from Lallemand Inc. and Viniflora CH11 from Chr. Hansen Holding AS) respectively for inoculation, and indigenous lactic acid bacteria (LAB) in a spontaneous MLF (Sp) (Figure 1). All fermentations (AF and MLF) were performed in biological triplicates. Statistical analysis of data was performed using ANOVA and the Tukey test with XLSTAT version 2022.5.1 software. A p-value of less than 0.05 was considered statistically significant.

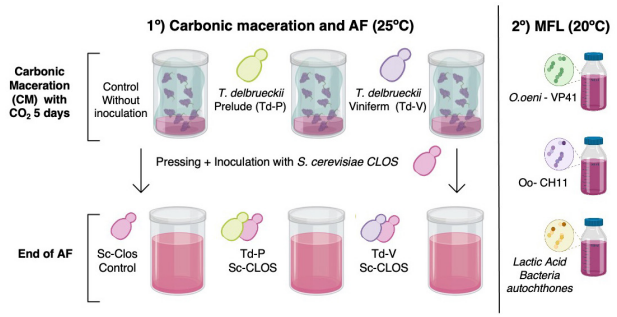



**FIGURE 1.** Experimental design.

## Results

### Effect of *T. delbrueckii* on wine colour

Interesting results were obtained regarding the colour and phenolic composition of the wines derived from inoculation with *T. delbrueckii* at the beginning of carbonic maceration. The presence of *T. delbrueckii* caused a significant increase in the concentration of anthocyanins in the final wines, especially with the TdV strain (Figure 2A). This higher concentration may be due to several causes, such as the greater oxidation of anthocyanins in the control wines, which took longer to begin FAL. On the other hand, this effect may also be associated with high pectolytic activity, which has been observed in certain non-*Saccharomyces* yeasts; however, this activity has not yet been demonstrated for *T. delbrueckii*. Differences were also found in pyranoanthocyanins, Vitisin A and Vitisin B (Figure 2B). These pigments play an important role in improving colour stability in wines, as they are less susceptible to pH changes and more resistant to discoloration caused by sulphur dioxide[3]. The control samples at the end of the CM were significantly different from those inoculated with *T. delbrueckii*, since no pyranoanthocyanins were detected. By contrast, both the TdP and TdV samples showed the presence of these pigments after CM. However, after AF, pyranoanthocyanins were detected in all wines, their concentrations being higher in wines with *T. delbrueckii* than in Control. During the CM phase, there were no significant differences in colour intensity (CI) between the different conditions. However, after AF, the control wines showed a significant reduction in CI, and it was possible to visually differentiate the control wines from the ones inoculated with *T. delbrueckii* (Figure 2C).

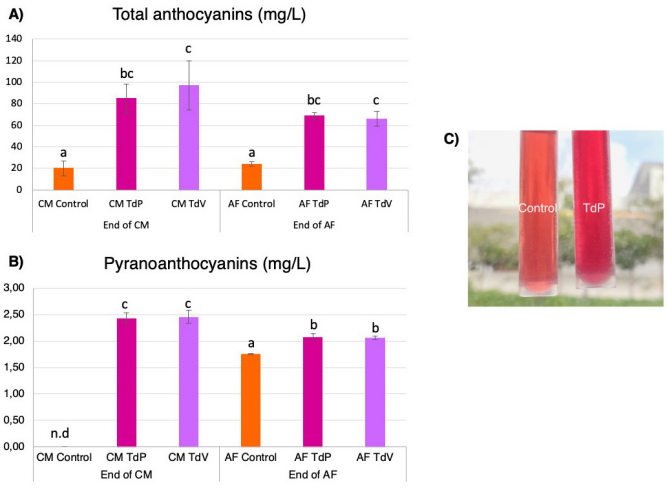



**FIGURE 2.** Analysis of phenolic compounds. A) Free anthocyanins analysed by HPLC, B) pyranoanthocyanins (Vitisins A and B), and C) differentiation of the wines with the naked eye: Control (Left) and wine inoculated with *T. delbrueckii* Prelude (Right). End of CM = sampling after carbonic maceration before inoculating *S. cerevisiae*; End of FAL = sampling after alcoholic fermentation. Control = the control fermentation; TdP and TdV = fermentations with *T. delbrueckii* Prelude and *T. delbrueckii* Viniferm, respectively. The different lower-case letters refer to significant differences between the groups ($p < 0.05$). All data are expressed as the arithmetic mean of three biological replicates ± standard deviation (n = 3).

### Effect of *T. delbrueckii* on the aroma and organoleptic properties of wines

Inoculation using the two strains of *T. delbrueckii* significantly influenced the volatile profile and general organoleptic characteristics of the wines.


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The differences were mainly observed after AF using *T. delbrueckii*, with an increase in the families of ethyl esters, higher alcohols, short chain fatty acids (SCFAs) and higher alcohol acetates. The compounds that increased the most were 1-propanol, 2-methylpropanol, isoamyl acetate, 2-phenylethanol acetate, ethyl decanoate, ethyl dodecanoate and isopropanol; these were characterized by the descriptors alcohol, wine, banana, rose, grape and leaves. The TdP strain showed a significantly greater increase in the concentrations of 2-phenylethanol, 2-methylpropanol, and isoamyl acetate than the TdV strain. Isoamyl acetate is a characteristic compound of CM wines[2] whose descriptor is banana. This compound reached concentrations of 0.62 mg/L (Control), 0.63 mg/L (TdP) and 0.47 mg/L (TdV) after CM without any significant differences. However, the TdP and TdV wines showed a significant increase in isoamyl acetate concentration after AF, reaching concentrations of 3.37 mg/L and 1.32 mg/L, respectively, relative to the Control AF wine at 0.58 mg/L (Figure 3A).

Finally, to comprehensively understand the impact of *T. delbrueckii* inoculation during MC, a sensory analysis was performed after AF. A triangular test was conducted by 15 trained judges, using black glasses to minimize visual bias. The results showed significant differences between Control and TdP, as well as between Control and TdV, but no distinction between TdP and TdV. Subsequently, a descriptive tasting of the wines was carried out after the AF: the TdV wine showed intense aromas of red fruits, grass and banana, as well as higher overall perception values, while in the case of the TdP wine, the banana aroma and the general perception improved compared to the control wine. Surprisingly, banana aroma was the only descriptor responsible for a significant difference between the wines with *T. delbrueckii* and the control wine (Figure 3B). This suggests that consumers associated a more pronounced banana aroma with the presence of *T. delbrueckii* during CM.

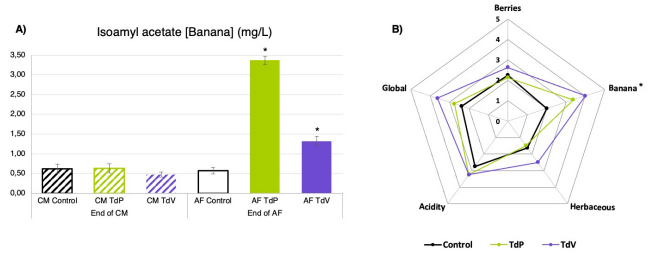



**FIGURE 3.** A) Isoamyl acetate concentration (mg/L), and B) spider web diagram of the sensory analysis of the wines after FAL. Asterisks (*) indicate attributes that showed significant differences (p value < 0.05). Control = control conditions fermented with Sc only; TdP and TdV = fermentations with *T. delbrueckii* Prelude and *T. delbrueckii* Viniferm, respectively. All data are expressed as the arithmetic mean of three biological replicates ± standard deviation (n = 3).

### Effect of *T. delbrueckii* on the malolactic fermentation performance

Once AF was completed, three MLF strategies were applied: inoculation with *O. oeni* strains OoVP41 and OoCH11, and spontaneous fermentation. Inoculation using *O. oeni* starter cultures was carried out in order to evaluate their potential under the competitive pressure of endogenous BAL.

Figure 4 shows the MLF kinetics of the three wine conditions with their respective MLF strategies. Inoculation with OoVP41 resulted in a shorter MLF duration than inoculation with OoCH11 and than in the spontaneous MLF. The TdP and TdV wines which were inoculated with OoVP41 completed the MLF two days earlier (i.e., within 8 days) than the control wines with the same strain (within 10 days). Previous studies under laboratory conditions[4] have also characterized the OoVP41 strain as a highly efficient fermentative strain, corroborating this result under these competitive conditions. The influence of *T. delbrueckii* on MLF was also observed when using the OoCH11 strain. Significantly greater differences were observed in the spontaneous MLF. The control wines took 7 days to initiate MLF, while TdP took 4 days and TdV only 3 days. Regarding the total time of MLF, TdP and TdV were also shorter: TdP lasted 15 days and TdV 16 days, which is notably less than Control, which required 20 days (Figure 4). The effect of *T. delbrueckii* on MLF is associated with various factors that contribute to improving the efficiency of *O. oeni*[1]. In the present study, despite no differences being found in ethanol or pH levels, the better performance of MLF can be attributed to a reduction in MCFAs (medium-chain fatty acids), known to be toxic to *O. oeni*[5], or to an increase in ammonium and mannoproteins. In addition to nutritional aspects, the increase in mannoproteins could be related to the reduction in MCFAs, since they can absorb these lipids, detoxifying the environment[6].

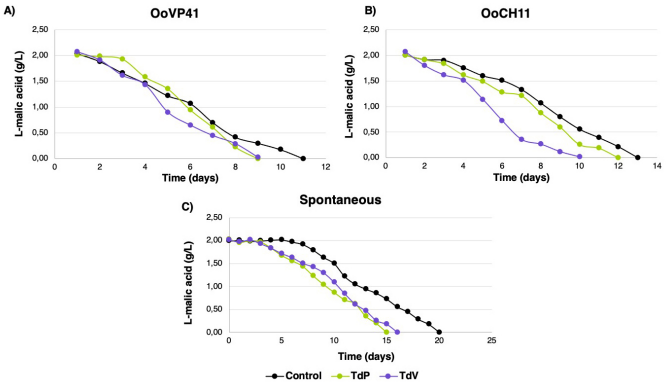



**FIGURE 4.** Consumption of L-malic acid by *Oenococcus oeni* strains during malolactic fermentation: A) MLF OoVP41, B) MLF OoCH11, and C) spontaneous MLF. Control = the control conditions fermented with Sc only; TdP and TdV = fermentations using *T. delbrueckii* Prelude and *T. delbrueckii* Viniferm, respectively. All data are expressed as the arithmetic mean of three biological replicates ± standard deviation (n = 3).

## Conclusions

Although in the present study the general physicochemical parameters were kept constant, the presence of *T. delbrueckii* significantly influenced the colour and aroma parameters, increasing the anthocyanin content and isoamyl acetate levels, especially with the TdP strain. Furthermore, *T. delbrueckii* significantly incremented other compounds associated with floral and fruity aromas.

The sensory evaluation revealed a tendency for increased red fruits, banana, and fresh grass aromas in TdV wines, and an enhanced perception of banana aroma and overall sensory attributes in TdP wines. Of these descriptors, banana aroma was the only one to show a statistically significant difference in the sensory analysis.

Regarding MLF, *T. delbrueckii* improved performance, especially in spontaneous MLF, starting earlier and requiring less time. Inoculation with OoVP41 led to a significant two-day reduction in the duration of MLF. These results highlight the potential benefits of using *T. delbrueckii* strains to improve MLF efficacy and the sensory attributes of carbonic maceration wines.

The approach used here could be especially useful in northern latitude wine regions, which are characterized by colder climates and higher acidity levels. MLF may become necessary in these regions to achieve the desired characteristics of their wines. Furthermore, inoculating with *T. delbrueckii* would help to enrich the sensory profile of these wines, guaranteeing high quality and distinctive characteristics. ■

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***Sources:*** *Sourced from the research article: "Torulaspora delbrueckii Improves Organoleptic Properties and Promotes Malolactic Fermentation in Carbonic Maceration Wines" (Fermentation, 2023).*

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