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Non-*Saccharomyces* as a tool for modulating wine quality and stimulating malolactic fermentation

Sourced from the research article "Impact of changes in wine composition produced by non-*Saccharomyces* on malolactic fermentation" (International Journal of Food Microbiology, 2021)[1].

>>> Recent research in non-*Saccharomyces* yeasts promotes their use as starter cultures in wine alcoholic fermentation together with *S. cerevisiae*. The use of these non-conventional yeasts can modulate the organoleptic profile of wines. However, it is unclear how they will modulate wines together with *Oenococcus oeni* after malolactic fermentation. In this article we discuss the main oenological consequences of these interactions and how malolactic fermentation can be stimulated using some of these non-*Saccharomyces* yeasts. <<<

Current oenological context

The winemaking industry must respond to current trends demanded by consumers, such as the improvement of aroma complexity leading to wine distinctiveness. In this sense, the use of non-conventional yeasts during alcoholic fermentation (AF) is a possible approach to differentiating wines. Those yeasts involved in the first stages of AF (i.e., non-*Saccharomyces* yeast) can modulate the organoleptic profile of wines.

The use of non-*Saccharomyces* yeasts focuses mainly on a few species, such as *Torulaspora delbrueckii* or *Metschnikowia pulcherrima*, which are provided in the form of starter cultures by oenological companies. These yeasts are related to the chemical modulation of wine in terms of aroma liberation, lowering ethanol concentration and increasing glycerol and mannoprotein concentration. The commercial strains of other species (i.e., *Lachancea thermotolerans*, *Pichia kluyveri* and *Schizosaccharomyces pombe*) have also been linked to some of these effects. Moreover, some non-*Saccharomyces* species/strains may play a bioprotective role against spoilage microorganisms[2].

Interestingly, some chemical changes caused by these non-*Saccharomyces* yeasts are linked to the mitigation of the harsh conditions present in wine for the development of malolactic fermentation (MLF)[3, 4].

MLF consists of the decarboxylation of L-malic acid to L-lactic acid, and the main species involved is the lactic acid bacterium *Oenococcus oeni*. MLF is related to an improvement in wine quality, since this biotransformation leads to a pH increase, the enhancement of organoleptic properties and microbial stabilisation[5]. MLF usually takes place after AF, so it is highly affected by the metabolism of the previous fermenting yeasts; as result, the inoculation of certain yeasts will have great impact on the development of MLF[6].

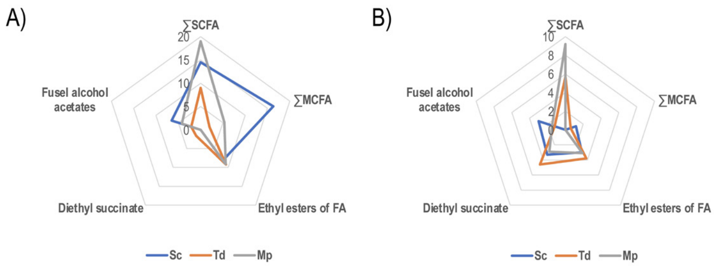

Figure 1. Concentrations of wine volatile compounds (mg/L) after AF grouped as family compounds. A) Macabeo wines. B) Cabernet Sauvignon wines. ∑, Sum; SCFA (propionic, butyric and valeric acids), MCFA (hexanoic, octanoic and decanoic acids), Ethyl esters of FA (ethyl hexanoate, ethyl octanoate, ethyl dodecanoate), Fusel alcohols (isobutanol, 1-butanol, 3-methyl-1-butanol, 1-hexanol, cis-3-hexen-1-ol, 2-phenylethanol, benzyl alcohol), Fusel alcohol acetates (isobutyl, isoamyl, hexyl and 2-phenylethanol acetates). Sc, Td and Mp correspond to *S. cerevisiae*, *T. delbrueckii-S. cerevisiae* and *M. pulcherrima-S. cerevisiae* fermented wines, respectively. Values shown are the mean of triplicates of the compounds with statistical differences.

S. cerevisiae is usually inoculated as starter culture to undergo AF; therefore, any change in the yeast used will somehow affect MLF performance. Thus, even if improvements are made to the organoleptic profile of wine after AF, other changes affecting MLF must be considered. Among the different species of non-*Saccharomyces*, *T. delbrueckii* and *M. pulcherrima* show the most promising results in terms of overall wine quality[4].

In this study[1], we evaluated the effect of the use of *T. delbrueckii* and *M. pulcherrima* strains on MLF in the production of white (Macabeo) and red (Cabernet Sauvignon) wine. Non-*Saccharomyces* were used in sequential inoculation with *S. cerevisiae* (after 48 h). The selection of this inoculation strategy was based on the results of a previous study[6] that showed more relevant changes in wine composition due to non-*Saccharomyces* strains when using this inoculation timing.

What can *T. delbrueckii* and *M. pulcherrima* do for *O. oeni*?

Generally, they can reduce the concentration of compounds related to an inhibitory effect upon *O. oeni*. In the wines obtained with these two non-Saccharomyces, a reduction in medium chain fatty acids (MCFA) was observed (Figure 1). These compounds can limit *O. oeni* growth and even decrease L-malic acid consumption[3]. Moreover, a significant 0.5 % (vol/vol) reduction in ethanol content was obtained in C. Sauvignon wines fermented with *M. pulcherrima*.

These two compounds, together with sulphur dioxide, are the most toxic to *O. oeni* in wine.

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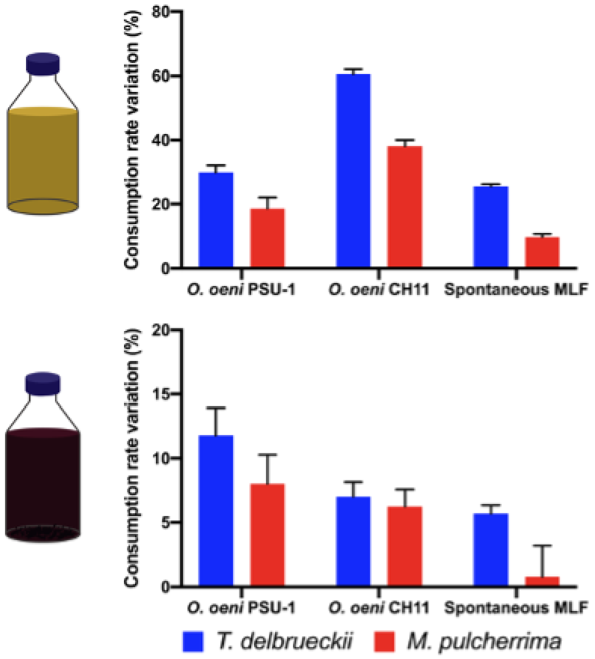

Figure 2. Percentage of increase in consumption rate of L-malic acid during MLF. A) Macabeo wines. B) Cabernet Sauvignon wines. Values represent the increase (%) in consumption rate of each MLF in non-*Saccharomyces* wines regarding to *S. cerevisiae* control wine.

Sulphur dioxide can be exogenously added, and it can also be produced by yeasts during AF. In this regard, *T. delbrueckii* wines had lower total SO_2 concentration (more than a 50 % reduction) than those just fermented with *S. cerevisiae* - even if not all the studied conditions reached the limit in toleration concentration for inoculated *O. oeni* strains.

■ What about the organoleptic profile?

The aroma of wines after AF was highly influenced by the use of non-*Saccharomyces*. The volatile profile was dependent on the inoculation strategy, whereby the use of non-*Saccharomyces* increased the concentration and type of aromas (Figure 1). After MLF, the wines were homogenised in terms of MLF strategy. Spontaneous MLF in white wine production thus resulted in the lowest aroma profile, while in red wine production it produced the most aromatic wines. Furthermore, the use of non-*Saccharomyces* somehow helped polyphenolic extraction and enhanced the anthocyanin concentration of red wines, with values ranging from 388 mg/L in *S. cerevisiae* wines to 451 and 426 mg/L in *T. delbrueckii* and *M. pulcherrima* wines respectively.

■ So can we promote MLF with the use of non-*Saccharomyces*?

Wine comprises a complex microbial environment in which nutrients are very limited. Under these stressful conditions, the reduction of inhibitor compounds directly affecting *O. oeni* may stimulate MLF. As result, those wines fermented with non-Saccharomyces had a higher consumption rate of L-malic acid (L-malic acid g/L per day) than in *S. cerevisiae* wines (Figure 2). In particular, *T. delbrueckii* wines showed the most favourable conditions for MLF performance, which is reflected in its quick MLFs. In addition, it promoted *O. oeni* diversity, which could be useful when a spontaneous MLF is desirable. We can therefore conclude that *M. pulcherrima* and particularly *T. delbrueckii* seem to promote MLF. Many compounds are related to this stimulatory effect; some of them are known, but there is still work to do in this field to unveil the whole picture of wine yeasts and *O. oeni* interactions. Moreover, since many of the metabolic changes are strain dependent, the yeast-bacteria strain compatibility is a key factor to obtaining successful results. ■

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