Use of non- *Saccharomyces* yeasts for the vinification of Tempranillo

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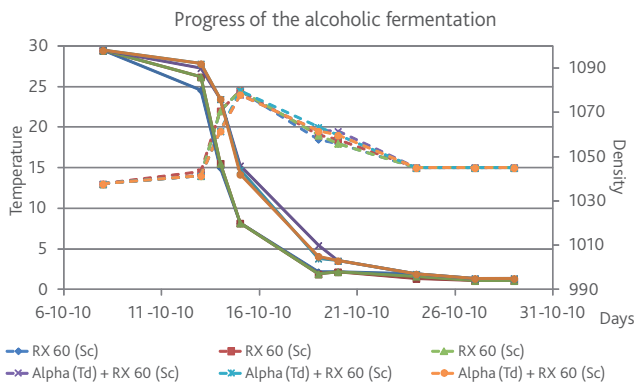
The initiation of spontaneous alcoholic ferments (wild ferments) does not result from only one species, nor from a specific yeast strain. The wine, after spontaneous alcoholic fermentation, is the sum of fermentative actions from different species which colonize the medium in sequential steps during the course of the fermentative process. As a result, the outcome and final product is unique for each fermentation (1).

Nowadays *Saccharomyces cerevisiae* dominates winemaking and is the main species present in most fermentation processes due to its ability to quickly adapt to various juice compositions and its resistance to alcohol. Although this species is not present at a high percentage at the onset and initial stages of fermentation, it becomes the dominant one towards the end of alcoholic fermentation.

Ongoing research and development of new fermentation agents (additives) which compliment but also protect varietal as well as territorial traits, without the loss of the wine's character, could be used as future tools in œnology (2, 3). Keeping this new innovative direction in mind, Laffort's research team has developed a new non-*Saccharomyces* strain, **ZYMAFLORE® ALPHA**, of the *Torulaspora delbrueckii* species. **ZYMAFLORE® ALPHA** has been investigated during its development phases in collaboration with leading wineries in, among others, the Spanish sector, nl. Winery Baigorri and Winery Iniesta.

T. delbrueckii is not alcohol-tolerant above 8-9 % (v/v).It is therefore necessary to perform a sequential co-inoculation with an alcohol tolerant yeast. That means 24-72 hours after *T. delbrueckii* fermentation activity starts, a *S. cerevisiae* strain of choice has to be inoculated. Comparative analysis (in triplicate) of pure *S. cerevisae* **(ZYMAFLORE RX60®)** cultivates and co-cultivates, *T. delbrueckii* **(ZYMAFLORE® ALPHA)** and *S. cerevisae* **(ZYMAFLORE RX60®)**, have been carried out in a semi-industrial scale experiment. The fermentation progress were similar for both trial modalities, the co-inoculation system being a little slower that the pure *S. cerevisiae* culture, as expected (Fig 1).

PCR analysis (RLPF-mtDNA: Restriction Fragment Length Polymorphism-mitochondrial DNA) at 12°Brix shows the coexistence of both species halfway through fermentation. Each strain is adapted to its niche environment at respective stages: *T. delbrueckii* at fermentation start while *S. cerevisae* finishes the alcoholic fermentation, when the alcohol level becomes too high (Fig 2).



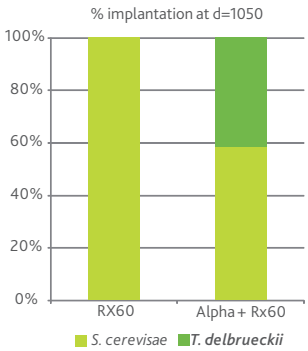
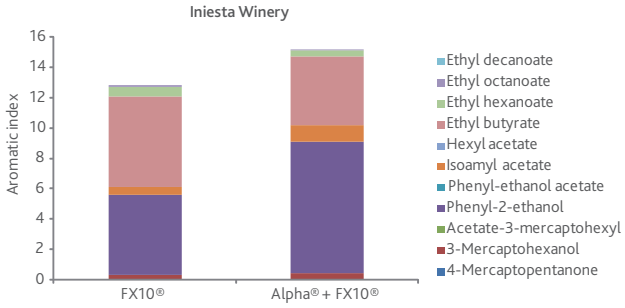


Fig. 1 and 2: Fermentation progress and implantation controls in pure and mixed T. delbrueckii **(ZYMAFLORE® ALPHA)** *and S. cerevisiae cultivates* **(ZYMAFLORE RX60®)**.

Trials carried out in wineries. The aim was to ascertain the ability of **ZYMAFLORE® ALPHA** to adapt to different terroir (affected by different pedo-climatic conditions) and to determine the characteristics of two different species (*S. cerevisae* and *T. delbrueckii*) in co-inoculation in order to enhance the differentiation of wine from the Tempranillo variety.

Results (3 months post alcoholic fermentation) show a positive increase of more than 20% in both cases for the aromatic potential. The most interesting was the differences observed for the obtained aromatic profiles. Two marked observations were the increment of 3-mercaptohexan-1-ol (the grapefruit attribute) and the significant increment of phenyl-2-ethanol (the floral attribute) (Fig 3 and 4). In addition, both wines presented a significant lower perception of astringency and tannins.



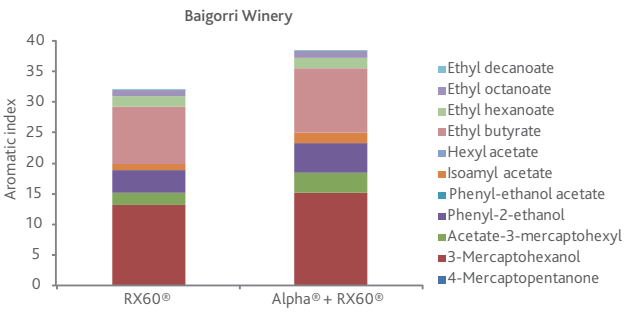


Fig. 3 and 4: Aromatic intensity (Aroma index) in wines inoculated with S. cerevisiae **(ZYMAFLORE FX10® and RX60®)** *and mixed T. delbruekii* **(ZYMAFLORE® ALPHA)** *and S. cerevisiae cultivates.*

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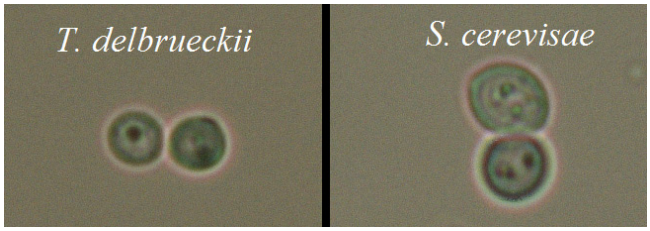
Conclusion. In the current global wine market consumers constantly demand new wine styles, without the loss of the terroir and grape variety characteristics. They want novel encounters and experience new sensations that will prickle their curiosity and enhance their overall wine appreciation. With these high expectations and a search for something innovative, the use of a yeast such as **ZYMAFLORE® ALPHA**, could present the winemaker with a new tool with which he could give an uniqueness to his wines that might fulfil the demands of the consumer.



Torulaspora delbrueckii is a non-Saccharomyces species, adapted and present in the grape must and the initial stages of fermentation. It displays the advantages of practically not producing acetic acid and acetaldehyde (2) and therefore having a very low production level of volatile acidity during the fermentative process. Morphologically similar to S. cerevisae, ZYMAFLORE® ALPHA, of the T. delbrueckii species, is a strain with low nutritional requirements and adapted to a wide range of temperature 16-26ºC.

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2. Maurizio Ciani and Francesca Comitini. Non-Saccharomyces wine yeasts have a promising role in biotechnological approaches to winemaking. Annals of Microbiology Volume 61, Number 1, 25-32, 2010.

3. Graham H. Fleet Wine yeasts for the future. FEMS Yeast Res 8 (2008) 979.

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