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Outcomes of wine fermentation with non-Saccharomyces yeasts in sequential inoculation: impact of the characteristics of the must

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Alcoholic fermentation, an essential step of winemaking, has been mainly controlled over the past 50 years through the use of selected starter cultures of *S. cerevisiae*. This is the safest way to ensure the completion of fermentation and to avoid undesirable off-compounds. The non-*Saccharomyces* yeasts, predominant in grape juice, are rapidly outcompeted by *S. cerevisiae* during fermentation because of their poor adaptation to increasing concentrations of ethanol and the lack of oxygen. Even though these species were used to be considered as spoilage microorganisms, their potential to improve the sensory quality of wines is now recognized. Although the behaviour of some non-*Saccharomyces* yeasts and *S. cerevisiae* were studied in mixed culture, the effects of must characteristics on the fermentation outcomes remained unknown.

Therefore, the impact of the main components of grape must, i.e. concentration of sugars, nitrogen and lipids, was studied in synthetic must thanks to a Box-Behnken experimental design. Non-*Saccharomyces* yeasts were tested in sequential inoculation with *S. cerevisiae*. The progress of the fermentations was monitored. Main metabolites and aroma compounds (higher alcohols, acetate and ethyl esters, acids and thiols) were measured at the end of the fermentation. Different aromatic profiles were obtained and the impact of each parameter as well as their interactions was assessed using the Box-Behnken model. Nitrogen appears to be the most influencing parameter. Results obtained in sequential inoculation were compared with those obtained in pure culture with *S. cerevisiae* to assess the benefits of non-*Saccharomyces* yeasts and to compare the differences in the response to must characteristics changes.

These results are of significance for the management of non-*Saccharomyces* yeasts in sequential inoculation for winemaking, and pave the way to more detailed metabolic and transcriptomic studies, in order to identify the metabolic and molecular basis underlying the phenotypic specificities of these species.