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Exploring the potentialities of *Metschnikowia* species during sequential wine fermentation

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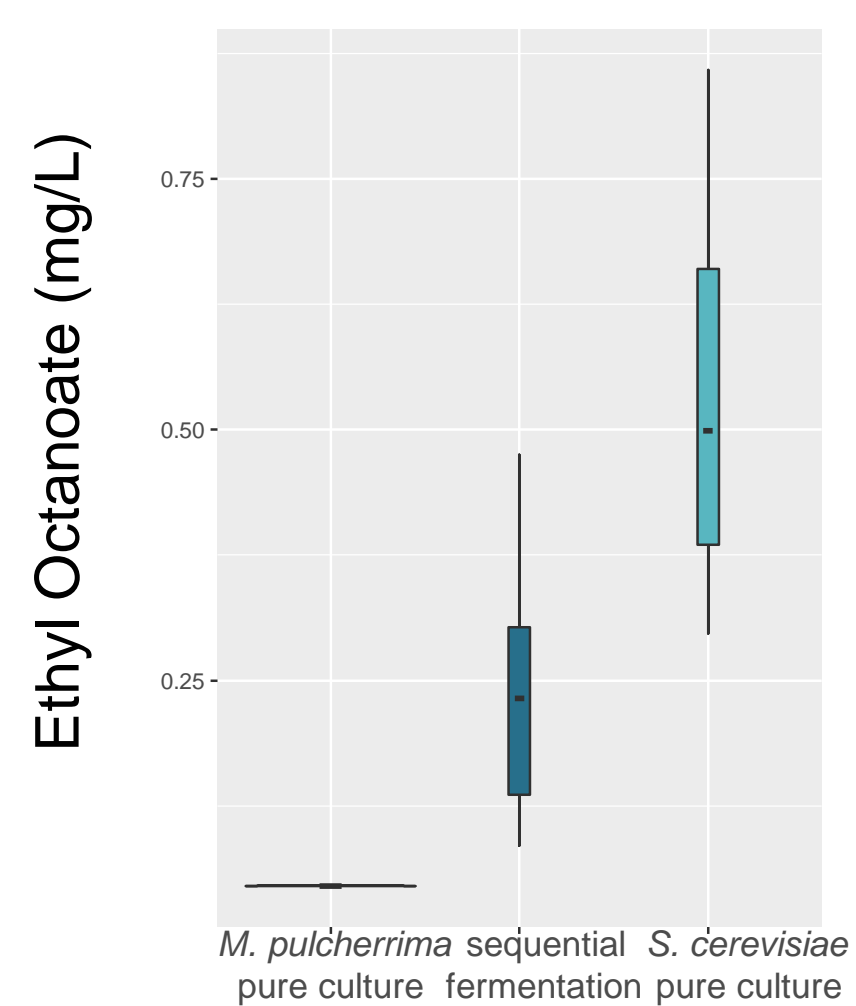
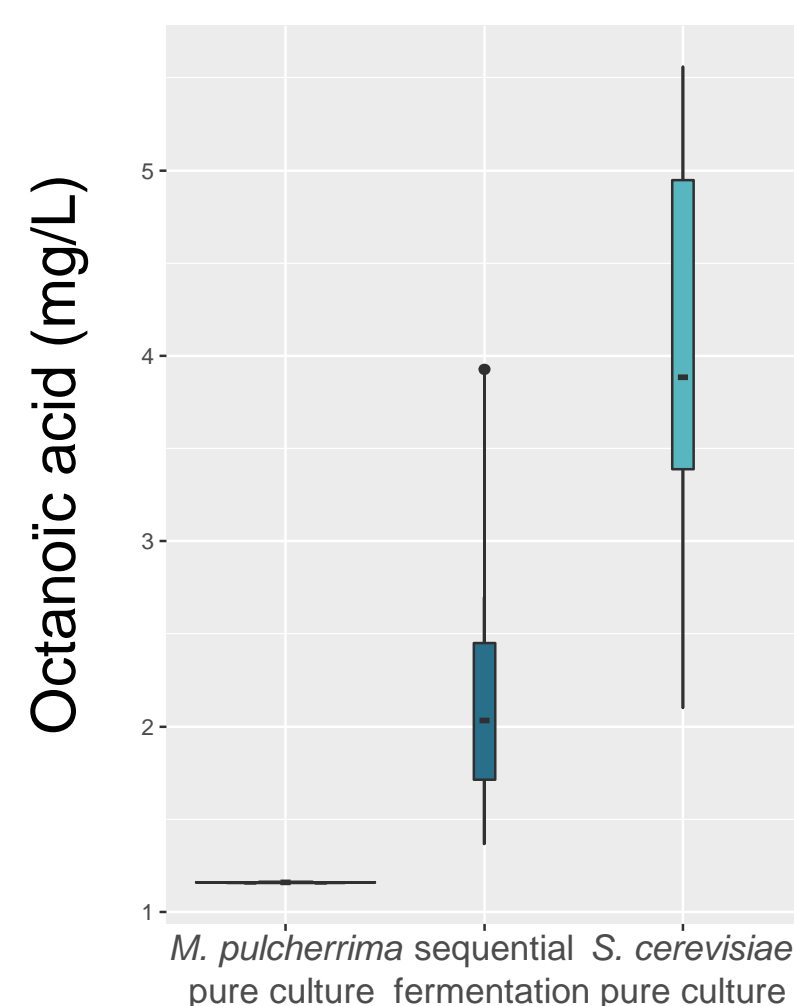
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Currently, in the winemaking sector, there is a renewed interest of the use of non-*Saccharomyces* species during the fermentation process. Long regarded as spoilage microorganisms, the potential of these yeasts, including *Metschnikowia pulcherrima* species, to improve the sensory quality of wines is now recognized. Due to their inability to complete the fermentation, *M. pulcherrima* strains must be used in combination with *Saccharomyces cerevisiae*, added in sequential or co-inoculation. However, the limited knowledge on their traits prevents to more widely gain the benefits and potentialities of *M. pulcherrima* during wine fermentation. To fill this shortcoming, this study investigated the specificities and the contribution of *M. pulcherrima* during sequential fermentation with *S. cerevisiae*.

production of medium chain fatty acids and ethyl esters

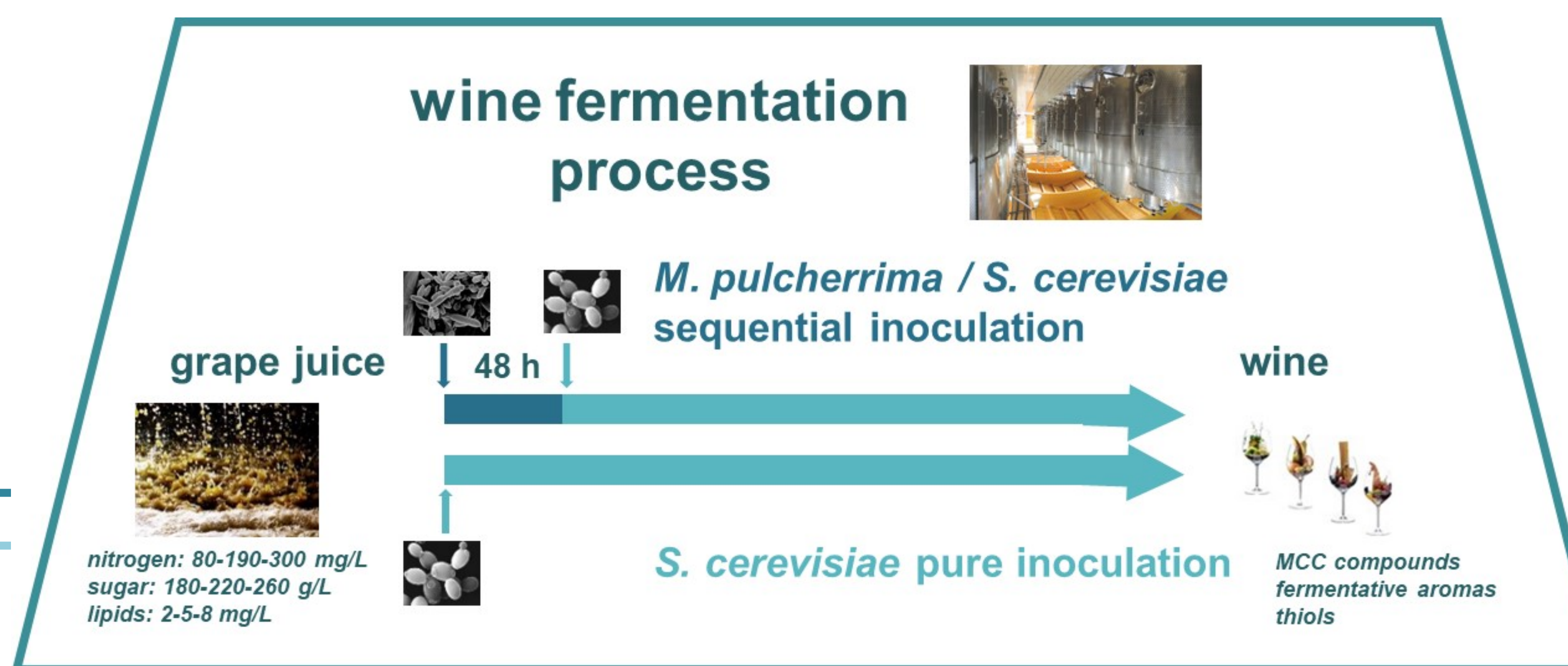
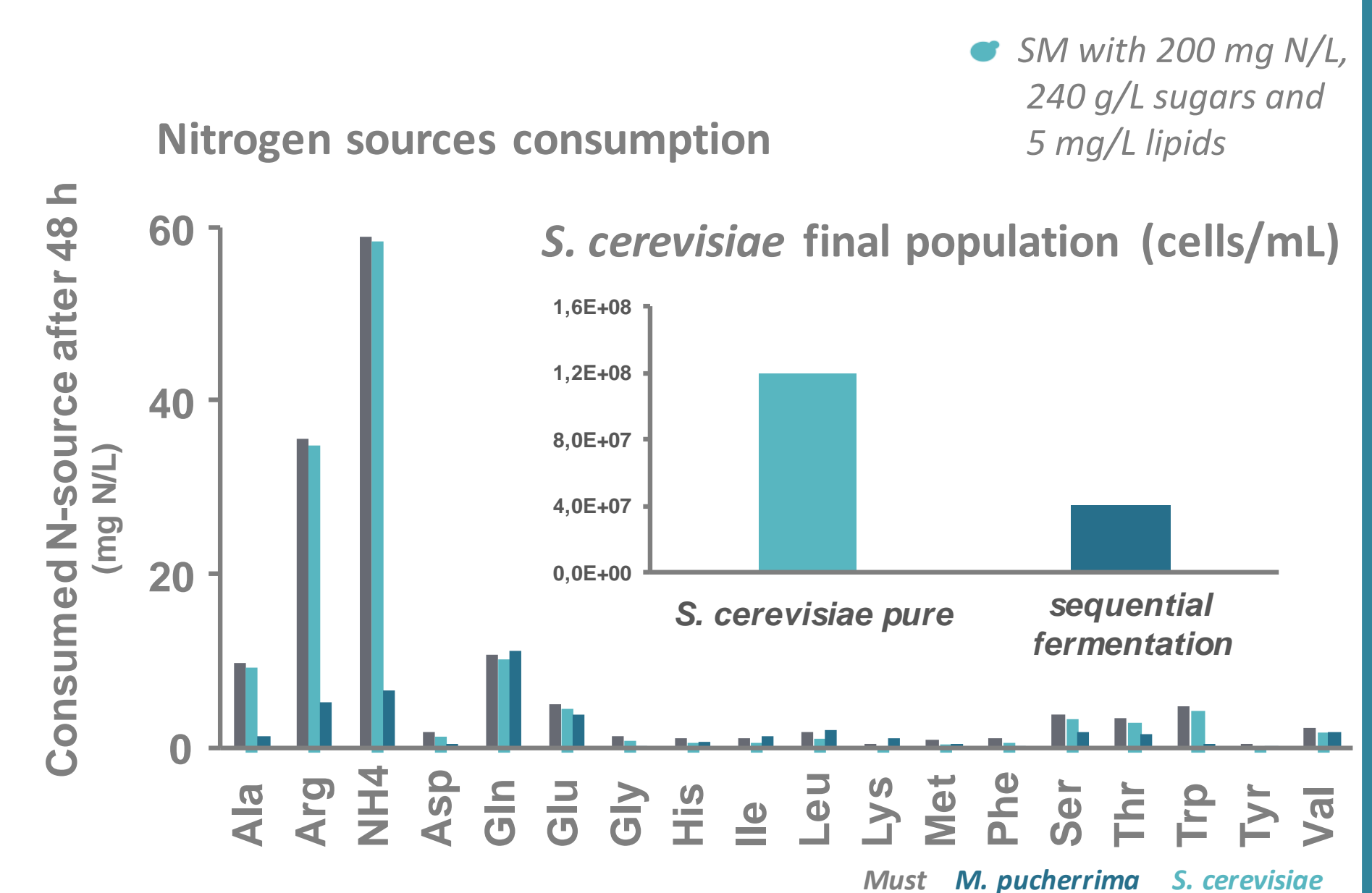
- Example of octanoic acid and ethyl octanoate.
- Similar profile observed with hexanoic, decanoic and dodecanoic acids and their ester derivatives



Compared with *S. cerevisiae* pure culture, sequential fermentation has a negative impact on:

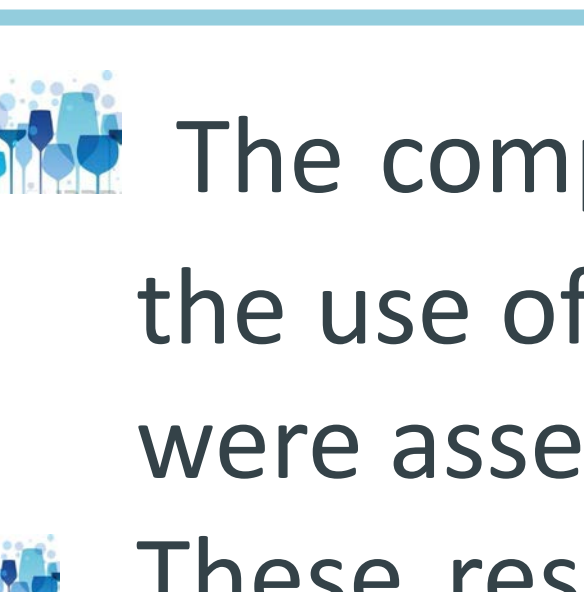
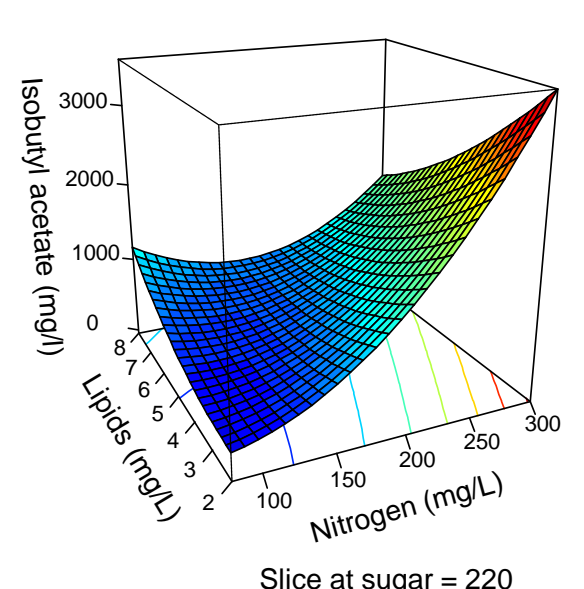
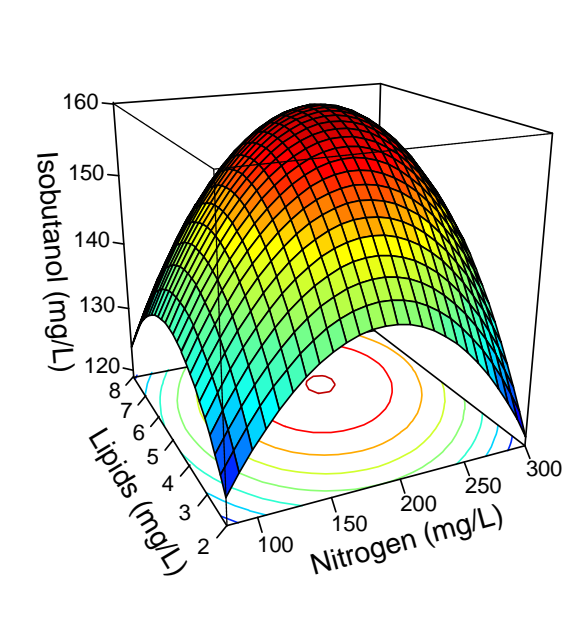
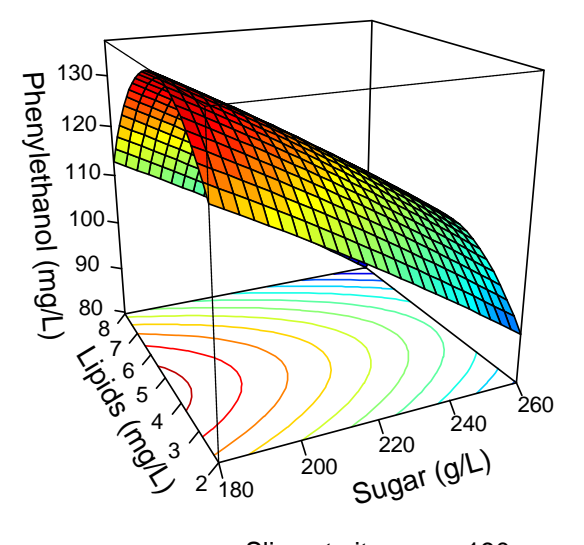
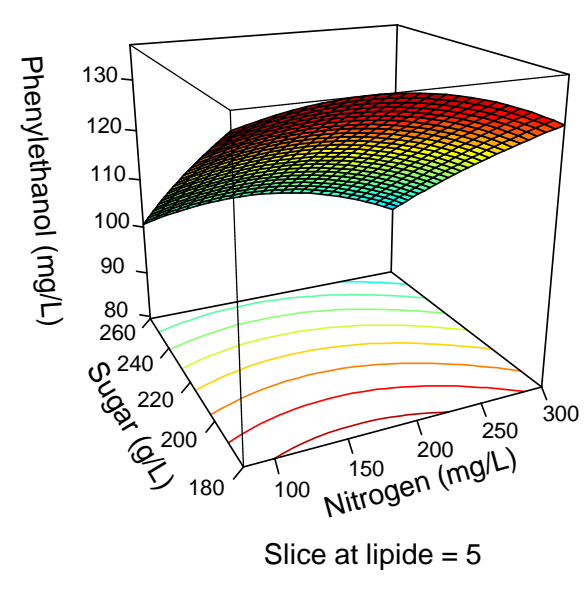
- the implantation of *S. cerevisiae*, due to the important consumption of glutamate, glutamine and branched amino acids of *M. pulcherrima*
- the fermentation rate, resulting in an increased fermentation duration
- the production of medium chain fatty acids and their ester derivatives

fermentation performances



production of higher alcohols and acetate esters

MP/SC sequential fermentation



Compared with *S. cerevisiae* pure culture, sequential fermentation has a positive impact on:

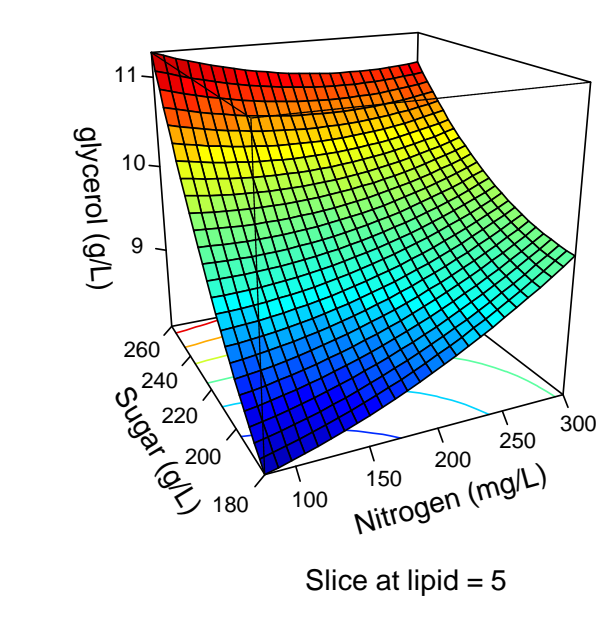
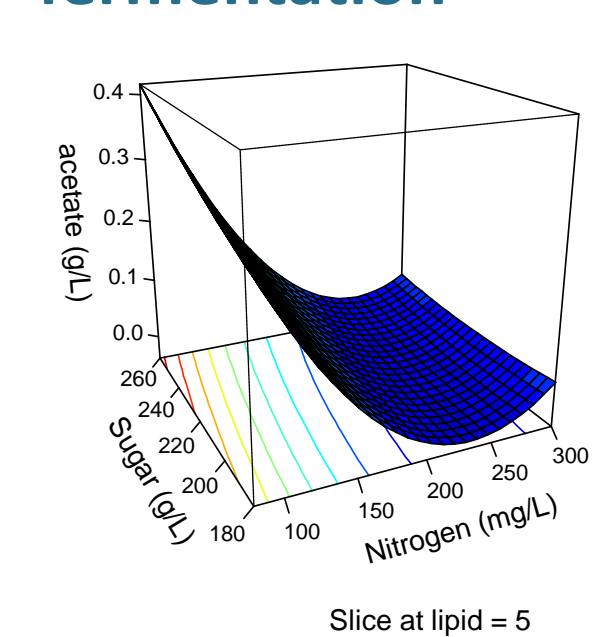
- the production of acetate esters (and some higher alcohols, depending on lipids, sugars and nitrogen availability)
- the production of acetate (decrease) and glycerol (increase)
- the production of positive thiols, which increases by a factor up to 6

The response to changes in nutrient availability may differ between MP/SC fermentation and SC pure culture

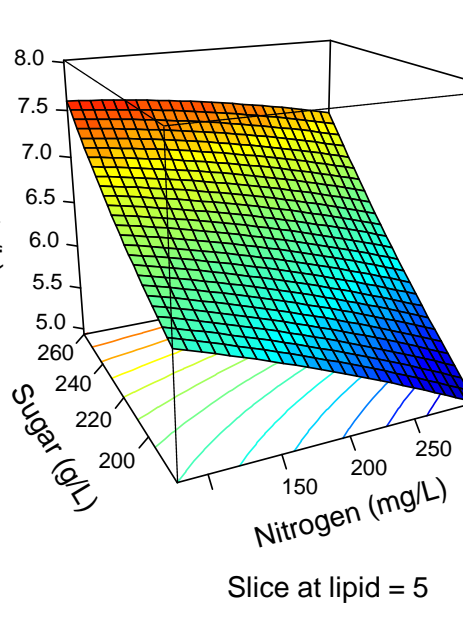
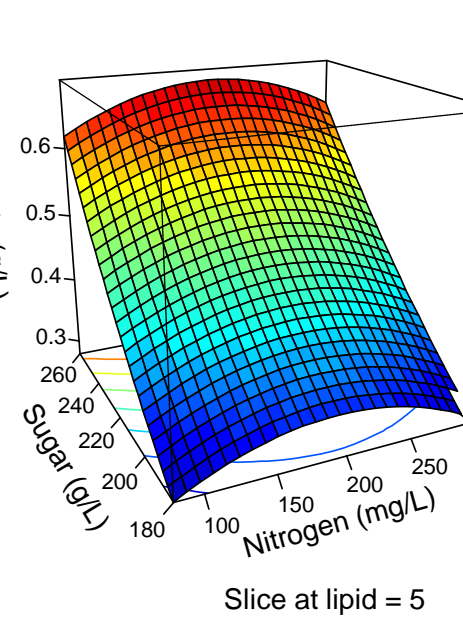
- strenght of the response
 - opposite variations
 - Interactions between factors
- depending on the compound

production of CCM compounds

MP/SC sequential fermentation

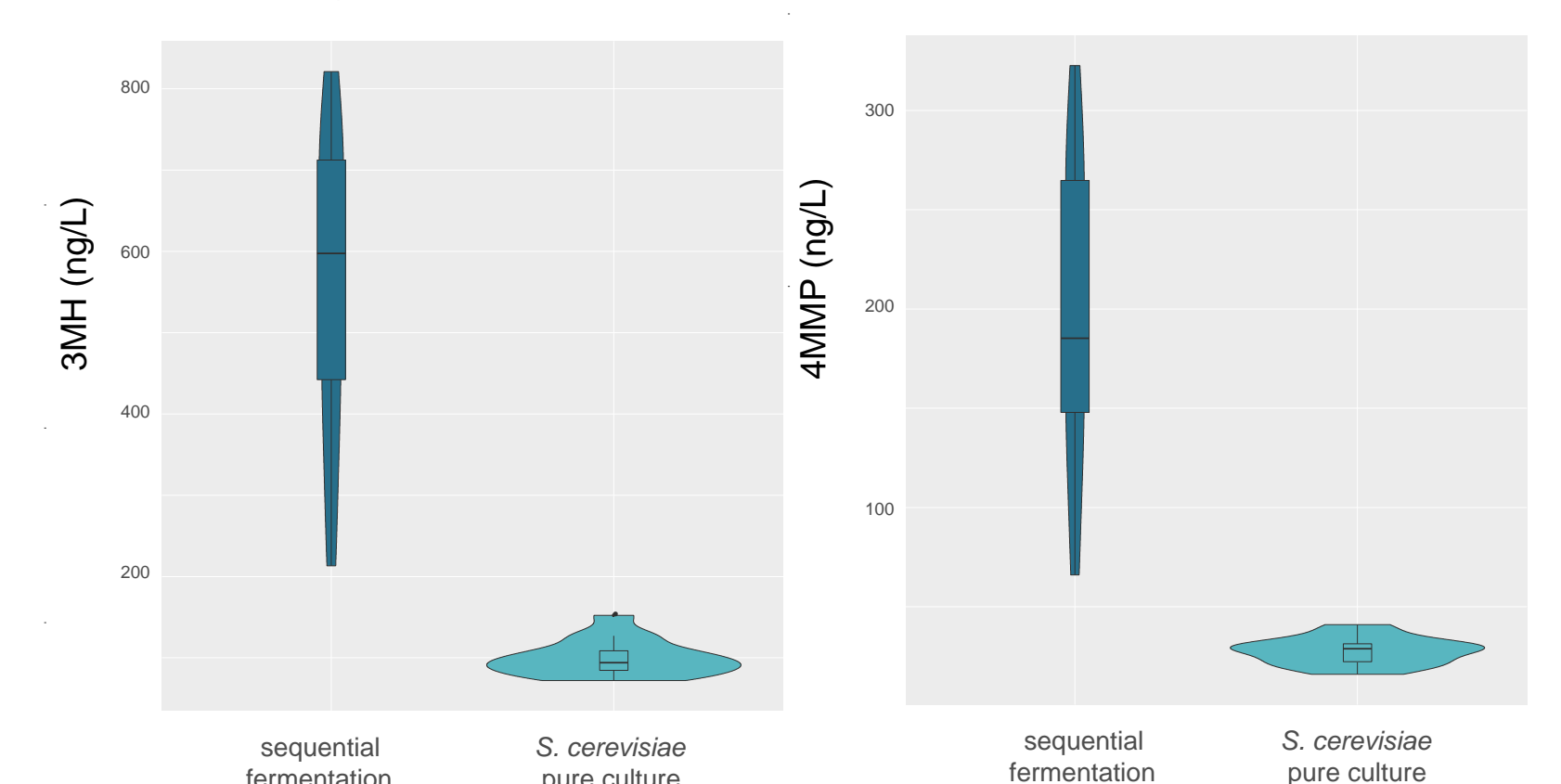


SC pure fermentation



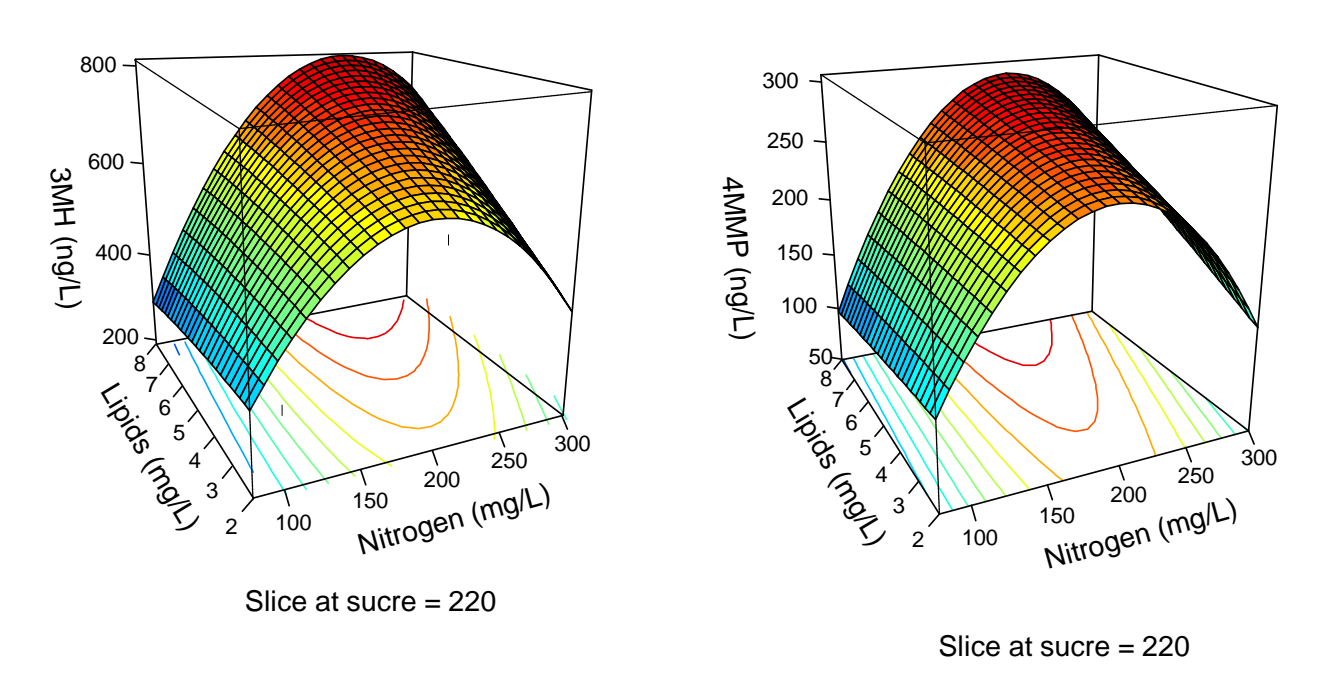
production of thiols

Thiols production in MP/SC sequential and SC pure fermentations



- No production of 3-MHA in *S. cerevisiae* pure culture
- Range of production of 3-MHA in sequential fermentation: 0-30 ng/L

Incidence of nutrients availability on thiols production in MP/SC fermentation



- Similar response of 3MH (3-mercaptohexan-1-ol) and 4MMP (4-mercapto-4-méthylpentan-2-one) as branched higher alcohols
- Similar profile of 3MHA (3-mercaptohexyl acetate) as acetate esters

The comparison between sequential inoculation and pure cultures pointed out the benefits but also the shortcomings of the use of *M. pulcherrima* in fermentation. Thanks to the Box-Behnken model, the impact and interaction of the parameters were assessed, showing that nitrogen was the most influencing nutrient on the sensory and organoleptic profile of wines. These results pave the way for the design of an improved management of sequential inoculation during winemaking to better exploit the potentialities of *M. pulcherrima* strains.