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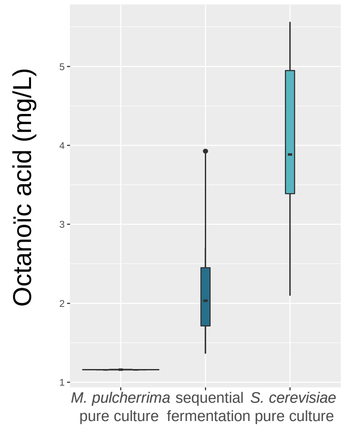
Exploring the potentialities of Metschnikowia species during sequential wine fermentation

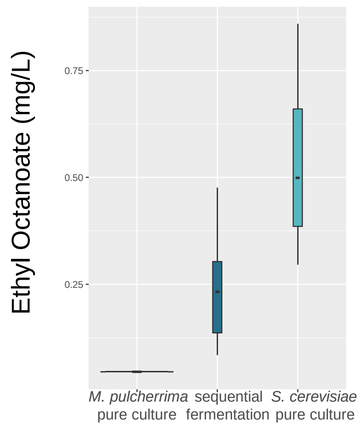
PAULINE SEGUINOT[1,2], ANNE ORTIZ-JULIEN[2], PASCALE BRIAL[1], CAROLE CAMARASA[1]

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 octanoïc acid and ethyl octanoate.*
- *Similar profile observed with hexanoïc, decanoïc and dodecanoïc acids and their ester derivatives*



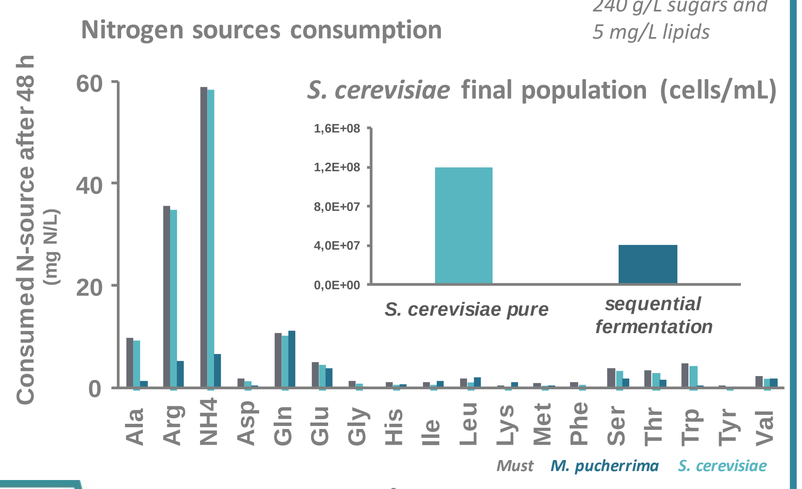


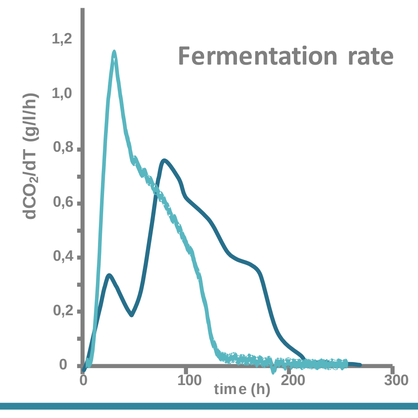
Compared with *S. cerevisiae* pure culture, sequential fermentation has a negative impact on:

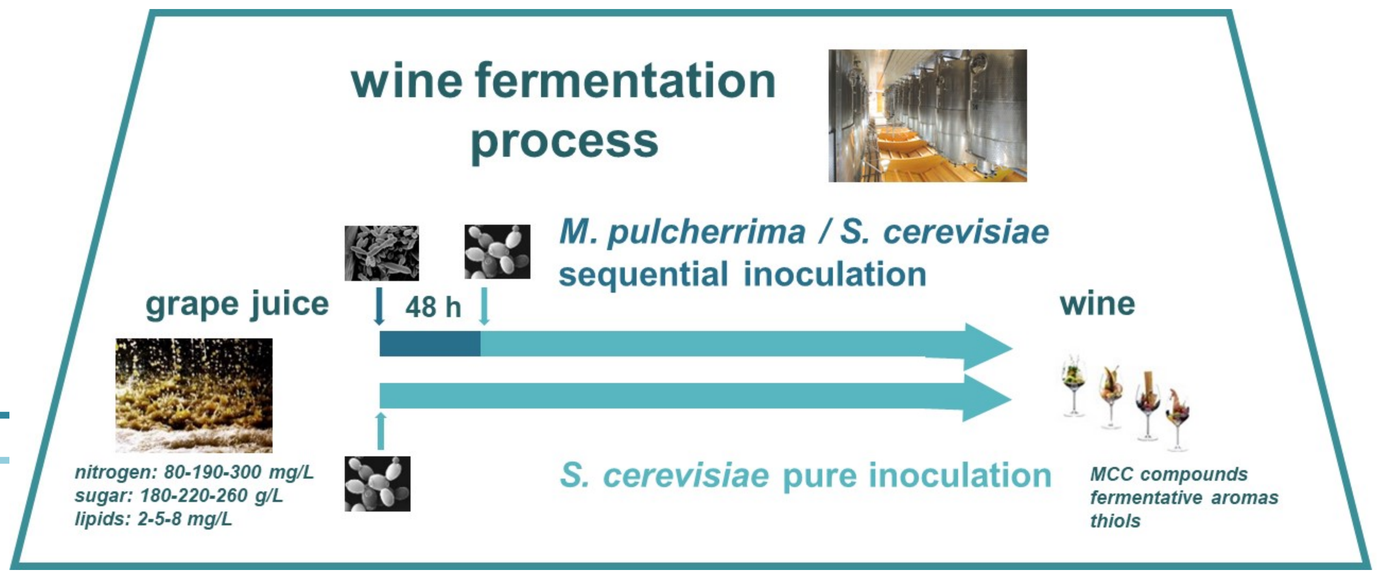
- the implantation of *S. cerevisiae*, due to the important consumption of glutamate, glutamine and branched amino acids of *M. pucherrima*
- the fermentation rate, resulting in an increased fermentation duration
- the production of medium chain fatty acids and their esters derivatives

fermentation performances

- *SM with 200 mg N/L, 240 g/L sugars and 5 mg/L lipids*



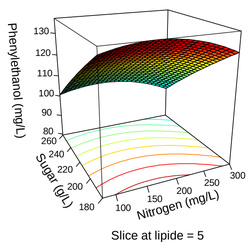




production of higher alcohols and acetate esters

MP/SC sequential fermentation

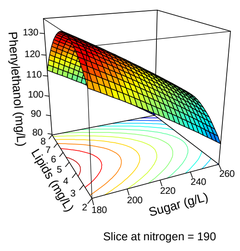
SC pure fermentation

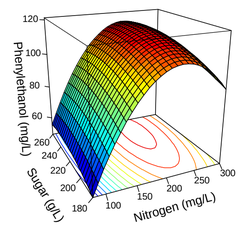


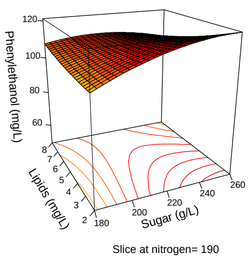
phenylethanol

Sugar: negative
Lipid: quadratic
Nitrogen: no impact

Sugar: no impact
Lipid: no impact
Nitrogen: quadratic



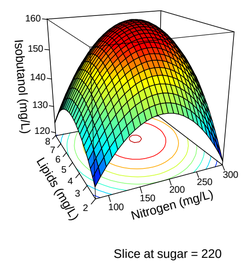


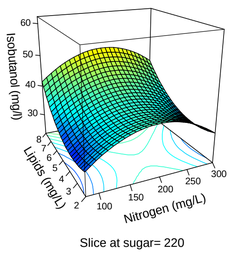


isobutanol

Lipid: quadratic
Nitrogen: quadratic

Nitrogen: quadratic

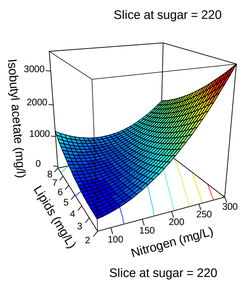


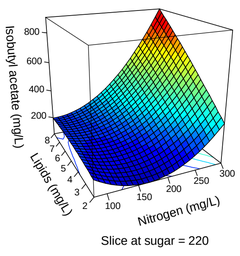


isobutyl acetate

Lipid: + low N
- high N

Lipid: no impact low N
+ high N





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

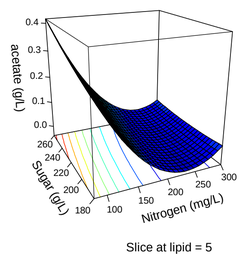
- strenght of the response
- opposite variations
- Interactions between factors

depending on the compound

production of CCM compounds

MP/SC sequential fermentation

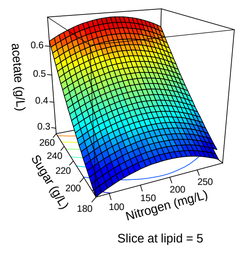
SC pure fermentation

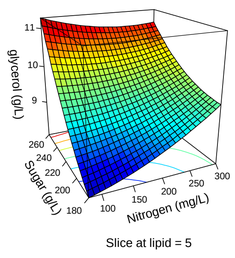


acetate

Sugar: no impact
Nitrogen: negative

Sugar: positive
Nitrogen: quadratic

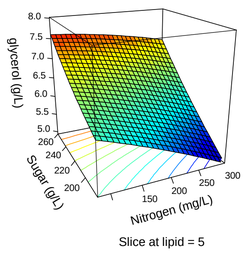




glycerol

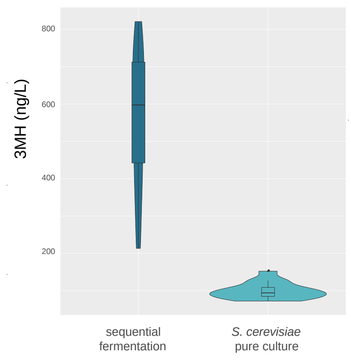
Sugar: postive
Nitrogen: + low sugar
- high sugar

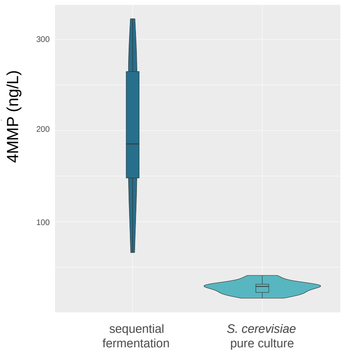
Sugar: positive
Nitrogen: negative



production of thiols

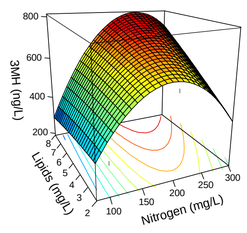
Thiols production in MP/SC sequential and SC pure fermentations

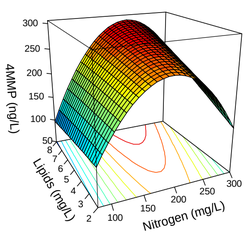


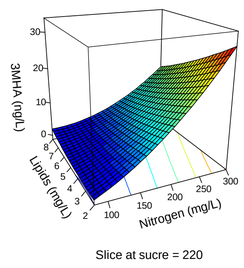


- *No production of 3-MHA in S.cerevisiae pure culture*
- *Range of production of 3-MHA in sequential fermentation: 0-30 ng/L*

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)l 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.

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