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# IMPACT OF NITROGEN SOURCES ON THE FERMENTATIVE KINETIC AND AROMA COMPOUNDS PRODUCTION BY NON-SACCHAROMYCES YEASTS

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Over the past 50 years, the completion of wine fermentation and the formation of undesirable off-compounds have been mainly controlled through the use of selected starter strains of *S. cerevisiae*. Little attention was paid to the non-*Saccharomyces* yeasts, predominant in grape juice but rapidly outcompeted by *S. cerevisiae* during fermentation. However, the potential of some of these species, thank to their ability to produce fermentative aromas, to improve the sensory quality of wines is now recognized even if the metabolic specificities of non-*Saccharomyces* yeasts remain ill-characterised. To fill this shortcoming that is a hindrance to an efficient use of non-*Saccharomyces* strains during wine fermentation, the aim of this study was to investigate to impact of the nature of nitrogen source on the fermentation performances and the production of volatile molecules of 2 non-*Saccharomyces* species.

## Material and Methods

Fermentation in synthetic media with **only one nitrogen source** (200 mgN/L) among 27 :

Ade, Ala, Arg, Asp, Asn, Cit, Cys, Cyt, GABA, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, NH<sub>4</sub>, Orn, Phe, Pro, Ser, Thr, Trp, Tyr, Urea, Val

Study of 3 **yeasts stains**, naturally present on grapes :

- *Torulaspora delbrueckii* (TD 291)
- *Metschnikowia pulcherrima* (MP 346)
- *Saccharomyces cerevisiae* (SC 09)

Fermentation in 15 mL-tubes – 1 AA per media  
**PhenoFerm** : automatic weighting device  
➤ Point each 4 h per tube to accurate determination of fermentation rate

Offline determination of metabolites

Measure of concentrations of 35 aroma compounds at the end of fermentation by GCMS

Residual nitrogen at the end of fermentation determined by NOPA analysis (amino acids) or enzymatic kit (NH<sub>4</sub>)

## Fermentation performances

**Fermentation profiles and kinetics parameters depend on both the strain and the nitrogen source**

Principal component analysis of fermentation parameters : lag phase (Tlag), maximal fermentation rate (Rmax), and time to reach the Rmax (Trmax).

**MP 346 : low performances regardless the nitrogen source**

Incomplete fermentation  
Low fermentation rates  
Short lag phase

**His, Lys, Cys : unable to support wine fermentation**

Incomplete fermentation  
Low fermentation rate  
Long lag phase

**SC 09 and TD 291 efficiently ferment sugars and most of the used nitrogen sources**

Complete fermentation  
High fermentation rates  
Long lag phase

### Efficiency of nitrogen sources to support wine fermentation

SC 09						TD 291						MP 346					
++		+		-		++		+		-		++		+		-	
Ala	Met	Cyt	Trp	Cys		Ala	Leu	Cyt	Pro	Ade		Ala	NH <sub>4</sub>	Ade	Tyr	Cys	
Arg	NH <sub>4</sub>	Gly		His		Arg	Met	Gly	Val	Cys		Arg	Orn	Cit		Cyt	
Asn	Phe	Pro		Lys		Asn	Met	Lys		His		Asn	Orn	Gly		His	
Asp	Ser	Thr		Lys		Asp	Orn	Ser		Cys		Asp	Ser	Gly		Lys	
GABA	Tyr	Ade		Thr		Cit	Phe	Thr		Trp		GABA	Thr	Phe		Trp	
Gln	Ure	Cit		Trp		Gln	Ure	Trp		Tyr		Gln	Pro	Met			
Glu	Val	Iso		Val		Glu		Iso		Iso		Glu	NH <sub>4</sub>	Met			
Leu		Orn										Leu	Arg	Val			

++ : best nitrogen sources + : average sources - : poor sources. In bold, nitrogen sources in the same category for the 3 strains

- In contrast to MP 346, TD 291 and SC 09 are able to achieve fermentation using most of amino acids as sole nitrogen source
- Many similarities in nitrogen sources preferences between non-*Saccharomyces* strains and *S. cerevisiae*
- Some differences :
  - Phe supports more efficiently TD 291 and SC 09 fermentation than MP 346 fermentation
  - Trp can not be used by MP 346 to carry out fermentation unlike SC 09 and TD 291
  - Lys sustains a moderate fermentation by TD 291, but not for SC 09 and MP 346

## Production of aroma compounds

**Production of volatile compounds during fermentation using a sole nitrogen source**

Aromatic profiles of TD 291 in fermentation on sole nitrogen source (normalized concentrations) :

Percentage difference between the production of aroma compounds by TD 291 in complete medium vs on medium with only valine :

Most of studied aroma compounds are produced irrespective of the nitrogen source  
➤ Compounds synthesized from MCC metabolites, regulated by total nitrogen availability

Huge increase in volatiles molecules that directly derive from  $\alpha$ -keto-isovalerate.  
Moderate increase of volatile compounds not derived from valine metabolism, as propanol  
➤ Impact of MCC

### Impact of the nitrogen source on the production of some aroma through Ehrlich pathway

Percentage differences between the production on complete medium and on sole nitrogen source medium

Overall, increase in the production of compounds derived from the nitrogen source  
Low ability of TD 291 to produce acetate esters except phenylethyl acetate  
In contrast, with MP 346 increase in the production of acetate esters except phenylethyl acetate. Low producer of acids and their ethyl esters derivatives

Similar responses to the nature of the nitrogen sources for the 3 species  
However, some species specificities, likely related to their genetic background

## Consumption of nitrogen sources

Percentage of residual nitrogen at the end of fermentation :

SC 09 : exhaustion of most of the nitrogen sources except Ser (15%), Lys (11%), His (85%), Glu (9%) and Cys (39%).  
TD 291 : complete consumption of Phe, Glu, Arg and Ala. Limited ability (residual nitrogen > 80%) to assimilate His and Gly. Higher consumption of Cys, Glu and Ser than SC 09.  
MP 346 : consumption between 47 and 98% of initial nitrogen. Higher consumption of Cys, His and Ser than SC 09.

Correlation between the amount of consumed nitrogen and fermentation performances. In particular, inability to sustain fermentation on poorly consumed nitrogen sources.

Distinct fermentative profiles were observed depending on the nitrogen source and the yeast strain. Yeasts had their own preferences in term of nitrogen sources that have shown diverse efficiency to sustain fermentative activity. Analysis of aroma compounds production according to the nitrogen source revealed a redistribution of carbon and nitrogen through the entire metabolism network, as the presence of a sole amino acid allows the production of all aroma compounds and not only those directly produced by the amino acid. Differences between the species have been highlighted, especially concerning the production of acetate esters. Nitrogen consumption is consistent with fermentation kinetics. These results provide new insights for the management of co-cultures of non-*Saccharomyces* and *S. cerevisiae*, emphasizing the importance of managing the nitrogen nutrition.

