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Dispersive Liquid-Liquid Microextraction for the quantification of terpens in wine

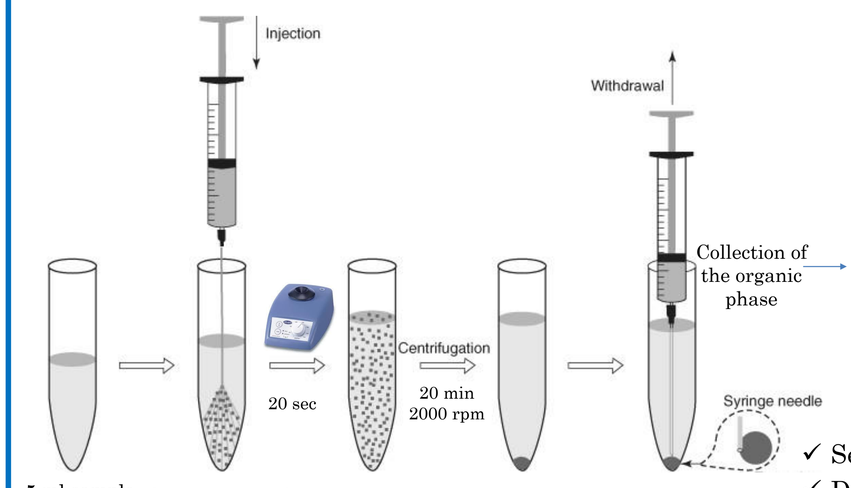
G. BERGLER[1,2], C. CAMARASA[1], M. BRULFERT[2], A. ORTIZ-JULIEN[3], V. NOLLEAU[1], C. PICOU[1], M. PEREZ[1], A. BLOEM[1]

Introduction

Wine is a highly complex mixture, composed by compounds belonging to different chemical groups and deriving from different pathways of the yeast's metabolism. Some of the volatile compounds are susbstantially produced by yeasts during enological fermentation (higher alcohols, acetate esters, …). Nonetheless, some other volatile compounds, such as terpen derivatives, are less produced, but can still have a great sensory impact on the wine. An analytical method, based on a Dispersive Liquid-Liquid Microextraction (DLLME) coupled to GC-MS, has been developed in order to quantify terpens in wines, compounds that can confer flowery notes. Then, this method has been validated, by studying potential matrix effects, linearity in enological range, repeatability, reproducibility, and by determining LODs and LOQs of the studied compounds. Finally, 40 yeasts strains were grown on natural must, and the validated analytical method was used to quantify the production of terpens by the yeasts during enological fermentation.

Development of the analytical method

Methodology : Sample preparation and GC-MS analysis

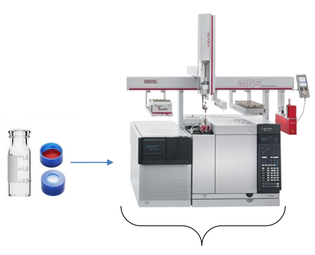


5 ml sample
20 µL ISTD (benzaldehyde -d6, 4-nonanol)

Optimization of the solvents volumes
870 µL acetone
500 µL CH_2Cl_2

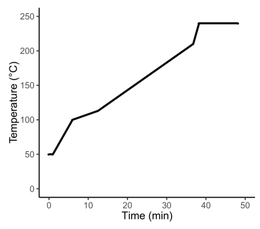
(from A. Zgoła-Grześkowiak and T. Grześkowiak, 2011)

Dispersive Liquid-Liquid MicroExtraction (DLLME)



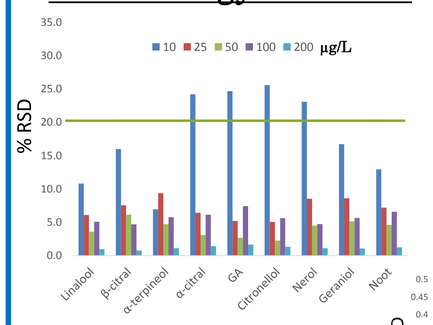
Selection of GC-MS parameters

- ✓ Selection of the column : DB-FFAP
- ✓ Determination of the mass spectra
- ✓ Optimization of the oven program



Validation of the analytical method

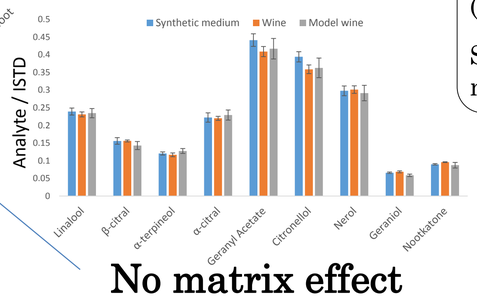
Methodology & results



Homoscedasticity of the data series

Study of the **matrix effect**

- 3 matrixes : model wine, white wine, fermented synthetic must
- Comparison of the ratios "Analyte/ISTD" on each matrix (1 concentration level)
- Statistical determination of the matrix effect (Fisher test)



Determination of recovery

No matrix effect

Validation carried out in fermented syntethic must

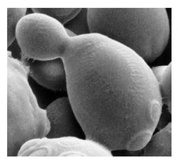
Lack-of-fit test

Analyte	Linearity parameters: Linear range (µg/L)	Calibration equation	Coefficient correlation (R^2)	Repeatability (% RSD): 25 µg/L	50 µg/L	Reproducibility (% RSD): 25 µg/L	50 µg/L	Limits: LOD (µg/L)	LOQ (µg/L)
Linalool	10-200	4.57x + 0.01	0.999	10.4	6.1	8.09 ± 0.02	5.38 ± 0.03	5.6	18.7
β-citral	10-200	3.62x + 0.01	0.998	11.7	9.8	10.31 ± 0.04	5.66 ± 0.05	9.9	32.9
α-terpineol	10-200	21.1x + 0.01	0.998	7.1	7.5	11.92 ± 0.04	5.78 ± 0.02	9.2	30.8
α-citral	10-200	5.71x - 0.002	0.998	12.8	11.7	7.12 ± 0.03	4.14 ± 0.02	11.3	37.6
Geranyl Acetate	10-200	8.84x - 0.01	0.998	8.9	7.3	8.24 ± 0.01	5.37 ± 0.03	5.7	19.0
Citronellol	10-200	5.88x + 0.003	0.998	9.6	7.2	8.31 ± 5.6.10^{-5}	5.13 ± 0.03	8.8	29.4
Nerol	10-200	6.81x - 0.001	0.998	10.1	7.8	7.54 ± 0.01	5.72 ± 0.03	8.8	29.2
Geraniol	10-200	1.42x + 0.002	0.998	14.5	5.7	8.94 ± 0.01	5.53 ± 0.02	5.9	19.7
Nootkatone	10-200	1.79x - 5.4.10^{-5}	0.998	8.8	9.0	8.81 ± 0.03	5.36 ± 0.06	10.5	35.1

Linear | Repeatable | Reproducible

Quantification of terpens after fermentations on a Chardonnay must

Experimental conditions



40 strains (duplicates) :

- 11 non-*Saccharomyces* strains
- 29 *Saccharomyces* strains

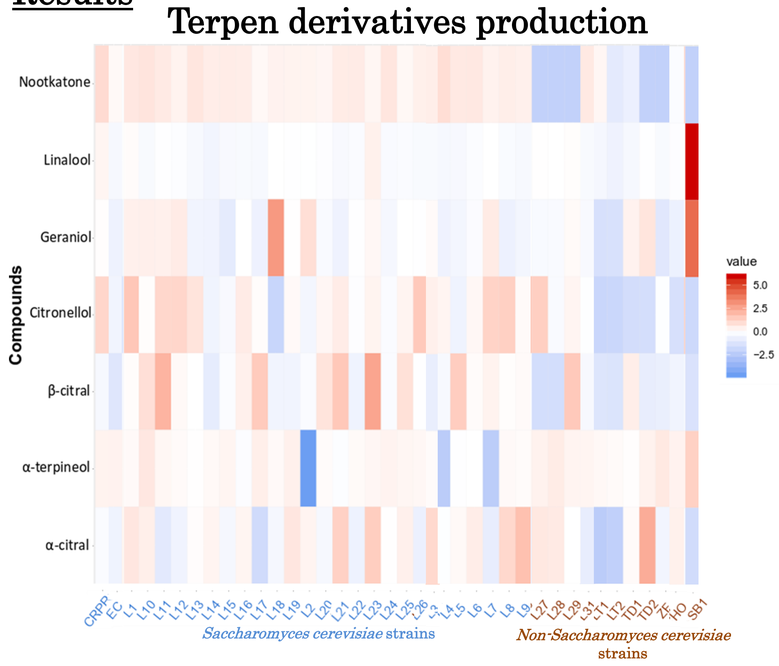
Alcoholic fermentation → 24°C

Must	Sugars (g/L)	Total Acidity (g/L H_2SO_4)	pH	Malic Acid (g/L)	Tartaric Acid (g/L)	Assimilable Nitrogen (mgN/L)	NTU
Chardonnay 2016	227.5	2.8	3.42	2.16	2.15	192	20 (adjusted to reach 4 mg/L of phytosterols)

Terpen derivatives quantitative analysis DLLME-GC/MS

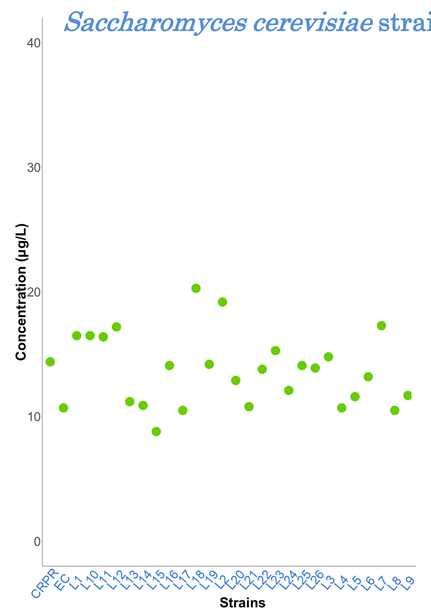
Results

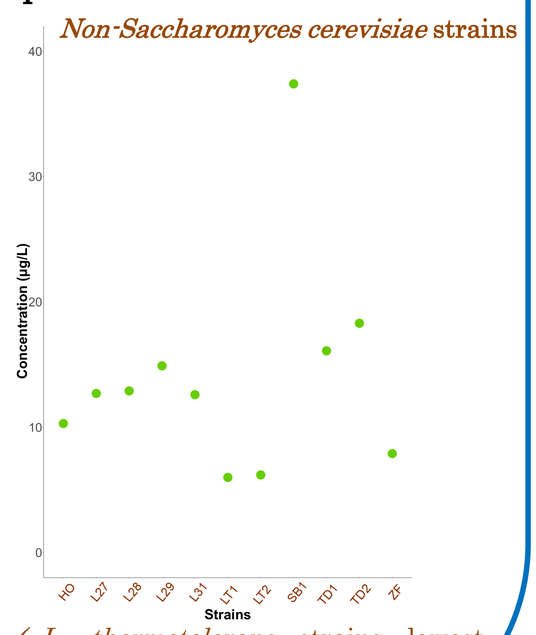
Terpen derivatives production



- ✓ Substantial production of monoterpenols
- ✓ Higher monoterpenols production by *S. cerevisiae* strains
- ✓ Non-*Saccharomyces* strains generally lower producers …
- ✓ … except for *S. bacillaris*, which produce highest amounts of linalool and geraniol

Example : Geraniol production





- ✓ Geraniol production between 8 and 21 µg/L
- ✓ Strain-dependent production
- ✓ *L. thermotolerans* strains lowest producers
- ✓ *S. bacillaris* highest producer of geraniol

Conclusion and perspectives

These data show the possibility of using Dispersive Liquid-Liquid Microextraction in order to quantify some compounds present in low concentrations in wines. After the optimization of conditions for the DLLME procedure and GC/MS parameters, the method proposed for the determination of terpens in white wine showed satisfactory linearity, precision and detection limits. This procedure is a simple, fast and inexpensive method that reduces the organic solvent use and extraction time. Therefore, it can be used for a high-throughput approach. This method has been applied to evaluate the production of terpen derivatives by wine microorganisms during the fermentation of a Chardonnay must. Interestingly, all the studied strains are able to produce terpen derivatives. One *Starmerella bacillaris* strain has stood out from the rest of the screening, and produces the highest amount of geraniol, linalool and α-terpineol. It could be interesting to estimate if the production is related to the presence of precursors in the must, or if it is due to their *de novo* metabolism.

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[1] UMR SPO, INRA, Université Montpellier, SupAgro, 34060 Montpellier, France
[2] Pernod Ricard, Paris, France
[3] Lallemand SAS, 31700 Blagnac, France