Unité de Recherche Œnologie

Optimization of -SH groups extraction from white wine lees using a design of experiments

Delphine Winstel [1] and Claudia Nioi [1]

[1] *Univ. Bordeaux, Bordeaux INP, Bordeaux Science Agro, INRAE, OENO, UMR 1366, ISVV, F-33140 Villenave d'Ornon, France*

Introduction

Glutathione is a natural tripeptide composed of L-glutamate, L-cysteine and glycine. This molecule is found in various foods and beverages. In particular, glutathione can be found in its reduced (GSH) or oxidized form (GSSG) in must, wine or yeasts[1]. Numerous studies have highlighted the importance of GSH in wine quality and longevity[2]. During winemaking, especially during aging on lees, GSH helps prevent the harmful effects of oxidation on the aroma of the wine[3]. In a recent study, results have shown that nitrogen- and sulfur-containing compounds are the main contributors to the antioxidant metabolome of white wine[4]. Nevertheless, the amount of GSH and -SH groups present in wine lees is often unknown and the choice of operating conditions (amount of lees and aging time) remains empirical. The aim of this study was to propose an optimized method to extract and quantify the potential of GSH from wine lees. In order to evaluate the main parameters affecting the extraction of -SH groups, the type of solvent, the extraction time and the solid-liquid ratio were investigated using a design of experiments (DOE).

Materials and Methods

Matrix and extraction protocol

Experiment plan conditions

- X_1: Ethanol concentration (%)
- X_2: Extraction time (min)
- X_3: Solid-liquid ratio ($g.L^{-1}$)

Parameters	X_1	X_2	X_3
Minimum	0	30	10
Maximum	100	180	30

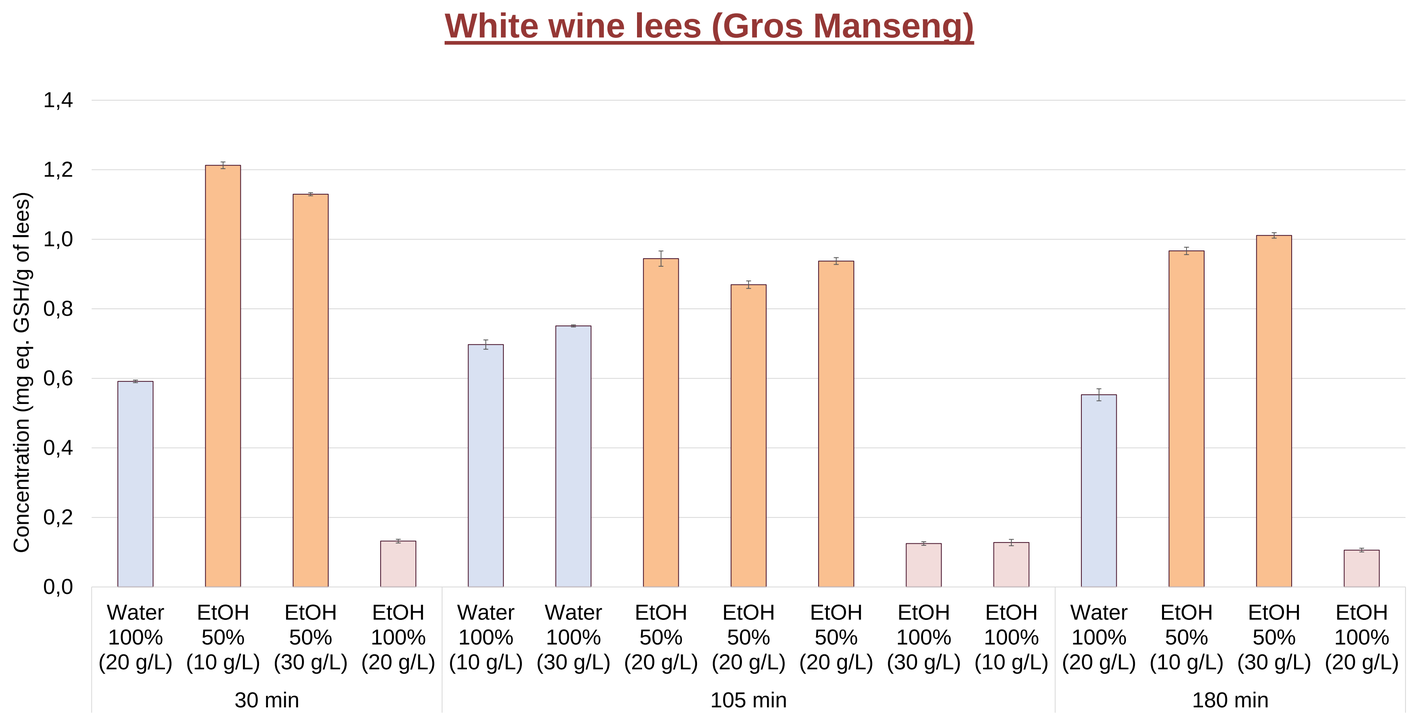
Design of experiments

- **Response Surface Methodology (RSM) by Box-Behnken (3-factor, 3-level, 1 solvent)**
- **15 total runs (including 3 center points)/lees**
- **Made on two different white wine lees (from Gros Manseng and Viognier winemaking)**

Results and Discussion

Implementation of the experimental plan

Run	Std. Order	X_1	X_2	X_3	Y ± SD (mg eq. GSH/g of lees)
1	1	0	30	20	0.591 ± 0.004
2	8	100	105	30	0.125 ± 0.005
3	2	100	30	20	0.132 ± 0.005
4	11	50	30	30	1.130 ± 0.004
5	12	50	180	30	1.011 ± 0.008
6	5	0	105	10	0.697 ± 0.013
7	6	100	105	10	0.128 ± 0.009
8	3	0	180	20	0.553 ± 0.017
9	10	50	180	10	0.967 ± 0.011
10	9	50	30	10	1.213 ± 0.010
11	**14**	**50**	**105**	**20**	**0.944 ± 0.022**
12	**15**	**50**	**105**	**20**	**0.869 ± 0.011**
13	4	100	180	20	0.106 ± 0.005
14	**13**	**50**	**105**	**20**	**0.938 ± 0.010**
15	7	0	105	30	0.751 ± 0.003



Optimization of results

Source of variations	Sum of squares	Degree of freedom	Mean square	F-values	Pr > F
ANOVA					
*Model	2.10	9	0.23	44.50	**0.0003***
Error	0.03	5	0.01		
Total adjusted	2.12	14			
Type III Sum of Squares					
X_1	0.55	1	0.55	105.50	**0.0002***
X_2	0.02	1	0.02	4.40	0.09
X_3	0.00	1	0.00	0.00	0.95
X_1^2	1.39	1	1.39	265.34	**<0.0001***
$(X_1)(X_2)$	0.00	1	0.00	0.01	0.93
$(X_1)(X_3)$	0.00	1	0.00	0.15	0.71
X_2^2	0.01	1	0.01	1.22	0.32
$(X_2)(X_3)$	0.00	1	0.00	0.78	0.42
X_3^2	0.05	1	0.05	10.38	**0.0234***
$(X_1)(X_2)(X_3)$	0.00	0	0.00	0.00	**<0.0001***

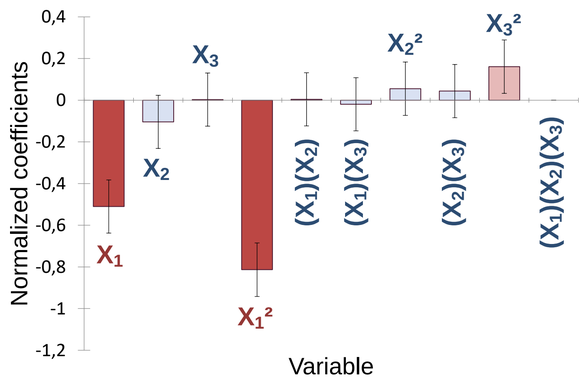
*Significant

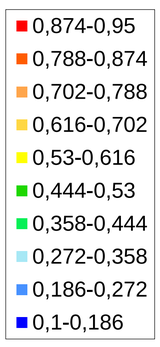
Types I, II, and III give the same results = data are balanced

$$Y = 0.92 - 0.26(X_1) - 0.054(X_2) + 0.001(X_3) - 0.61(X_1^2) + 0.003(X_1)(X_2) - 0.014(X_1)(X_3) + 0.042(X_2^2) + 0.032(X_2)(X_3) + 0.12(X_3^2)$$

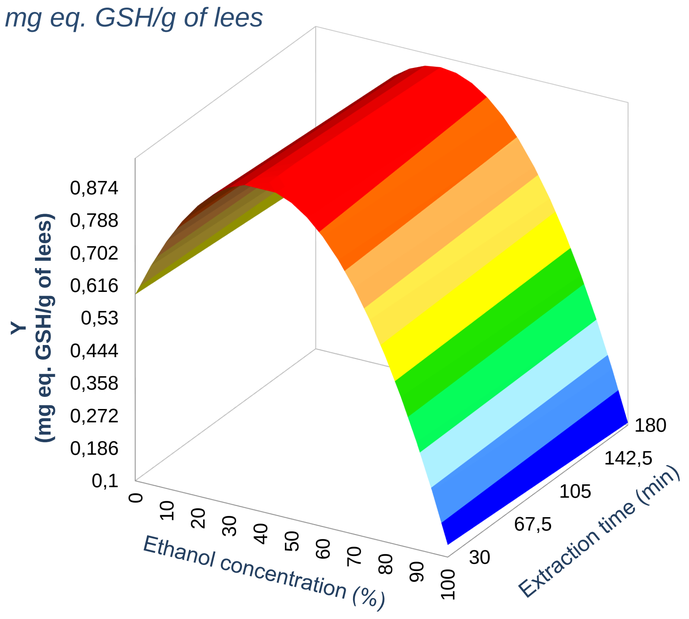
Optimal conditions: **EtOH 50% // 30 min // 10 $g.L^{-1}$**

Predicted concentration: 1.14 mg eq. GSH/g of lees





R^2 0.996



Contribution of variables: X_1 (96%) ; X_2 (4%) ; X_3 (0%)

Conclusion

Using DOE to evaluate and optimize extraction processes yields the most useful information from fewer experiments, thereby minimizing costs and maximizing desired responses. In this study, a RSM by 3-level Box-Behnken Design was applied to improve the extraction of -SH groups using 3-factors from white wine lees. The results showed that the main factor influencing the extraction efficiency was the ethanol concentration. Furthermore, the experimental device was validated and its predictive capacity was adequate. The optimal condition for the highest -SH groups content was obtained by using a 50% hydro-ethanolic solution at 10 $g.L^{-1}$ for 30 minutes. This new methodology is easier and cheaper than other methods to perform the extraction of -SH groups from wine lees because it does not require expensive reagents or large amounts of organic solvents. This approach will be extended to the analysis of oenological products as yeast derivatives. Finally, this study offers promising perspectives since by-products such as lees can provide new natural products to the food industry, with safer and better antioxidant qualities against oxidative damage.

ISVV INSTITUT DES SCIENCES DE LA VIGNE ET DU VIN BORDEAUX AQUITAINE — université de BORDEAUX

1. Foyer, C. and Noctor, G., *Plant Cell and Environemental*, 28(8), p. 1056-1071, **2005**
2. Pons, A. *et al.*, *American Journal of Enology and Viticulture*, 66(2), p. 187-194, **2015**
3. Lavigne, V. and Dubourdieu, D., *In 13th International Enology Symposium*, **2002**
4. Romanet, R. *et al.*, *Antioxidants*, 9(2), p. 115, **2020**

anr « ValoLi »
Chaire Denis DUBOURDIEU Qualité et identité des vins