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Development of a methodology for the determination of glutathione and oxidized glutathione in wine lees and yeast derivatives

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Wine lees and yeast derivatives are used in winemaking to improve and conserve organoleptic quality. Among the various quality benefits of aging white wines on lees, protection against oxidation due to the release of glutathione (GSH) has been clearly demonstrated. More recent studies show that GSH-rich yeast derivatives can also protect wines against oxidation. Precise determination of the GSH content of these yeast matrices is thus essential to better control of their use in winemaking. This paper proposes an optimized methodology for the extraction and analysis of glutathione in its reduced and oxidized forms in both matrices, wine lees and yeast derivatives.

Background

Glutathione is a tripeptid found in various foods and beverages, including grape must and wine, in its reduced (GSH) or oxidized (GSSG) states[1]. During aging on lees, the GSH released limits wine oxidation phenomena[1 2 3]. Pre-fermentation maceration, gentle pressing under inert conditions, aging on lees and the use of glutathione-enriched yeast derivatives (YDs) are good practices for protecting and increasing GSH levels in wine[2 3 4]. YDs are preparations obtained from *Saccharomyces cerevisiae* and/or non-*Saccharomyces* yeasts[5]. Recent studies have demonstrated the antioxidant properties of these compounds[6]. The protection against oxidation observed in wines treated with YDs, as in the case of aging on lees, seems to be linked to their ability to consume O_2 and to the presence of GSH[4]. However, while the concentration of GSH in wine can be measured using validated methods, the concentration of GSH in wine lees and YDs is often unknown. There are currently no specific methods in the literature for the extraction and analysis of total glutathione in wine lees. In contrast, the glutathione in yeast derivatives can be quantified by colorimetric methods using a reference range of pure glutathione solutions. However, the methods reported in the literature vary considerably, often meaning that results cannot be compared. As a result, we have developed an optimized method for assessing the GSH potential of both these yeast matrices. GSH and GSSG concentrations were then determined in various samples of wine lees and yeast derivatives. The results presented in this paper are a summary of work recently published in the journal *Food Chemistry*[7].

Experimental method

The study was based on 23 samples of white wine lees collected from wine estates in the Bordeaux region. The samples were taken after the alcoholic fermentation (after 1 week) or after aging (6-12 months). The YDs (15 samples), naturally rich in reducing compounds including GSH, were supplied by their manufacturers. After extraction, the samples were freeze-dried and analyzed by liquid chromatography-high resolution mass spectrometry (LC-HRMS). The chromatographic analysis methodology is described in the original article by Winstel *et al.*[7].

TABLE 1. Optimum glutathione extraction conditions for white wine lees and yeast derivatives.

Sample	Ethanol (%)	Extraction time (min)	Solid-liquid ratio (g/L)	GSH extracted
Lees (µg/g lees)	0	105	10	4.30
Yeast derivatives (YDs) (mg/g YD)	50	30	10	49.20

Development of a method for quantitation and extraction of GSH and GSSG

To study the composition of complex matrices such as wine lees and YDs, powerful and reliable instruments are essential. LC-HRMS is suitable for GSH quantification due to its wide dynamic range and precise measurement of mass.

Thus, after extraction of GSH from wine lees and YDs, chromatographic conditions were optimized to improve separation of GSH and GSSG and enable their quantitation in both yeast matrices.

Following the development of the analytical methodology, GSH extraction was optimized. An experimental plan was thus drawn up, including solvent type, extraction time and solid-liquid ratio, to assess the combined impact of these factors on GSH extraction. Optimum conditions (Table 1) for extraction of all the GSH were obtained using a 50 % ethanol/water solution at 10 g/L for 30 minutes for yeast derivatives and 100 % ultrapure water at 10 g/L for 105 minutes for wine lees. This optimization study made it possible to quantify the GSH potential in both matrices.

Quantitation of GSH in different white wine lees samples

Samples from different cellars, grape varieties, yeast strains, and vintages, and including free-run and press wines, were analyzed to assess the range of GSH and GSSG concentrations that can be found in lees as a function of winemaking practices (Figures 1A and 1B). As shown in Figure 1A, concentrations ranged from 0.3 to 7.7 µg/g and from 0.3 to 34.4 µg/g for GSH and GSSG respectively. For some samples of lees (1 to 4 and 7), GSH was present in higher concentrations than GSSG, while the opposite was observed for samples 5-6 and 8 to 15. A higher concentration of GSSG could be explained by the fact that the lees were not protected from oxidation (vinification without addition of SO_2). The sensitivity of glutathione to oxidation during vinification has already been demonstrated[3] and the final concentration of GSH in the lees is thus dependent on winemaking practices.

GSH and GSSG were also quantified in lees samples from the same vineyard and must, but fermented using different yeast strains (Figure 1B). Variations in concentration were observed from one sample to another, ranging from 0.8 to 913.5 µg/g and from 72.6

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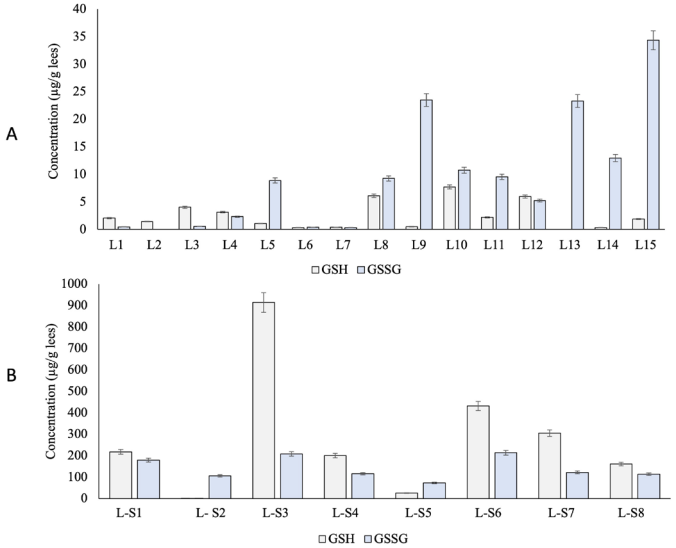


FIGURE 1. Concentration of GSH and GSSG in 15 lees samples (L1-L15, taken from the cellar 1 week after alcoholic fermentation or after 6 months of aging) from white wine from different estates (Figure 1A) and 8 lees samples from the same estate but fermented with different yeast strains (L-S1 to L-S8, taken 1 week after alcoholic fermentation) (Figure 1B). Extraction conditions: lees in water at 10 g/L for 105 min.

to 213.7 µg/g for GSH and GSSG respectively (Figure 1B). Lees taken from wine fermented with yeast strain 3 contained the highest concentration of glutathione. These results clearly demonstrate the importance of choosing the right fermentation yeast to preserve GSH and particularly in the case of vinification without sulfite addition.

Quantitation of GSH in different white wine lees samples

Currently, GSH in yeast derivatives is generally determined using colorimetric methods, the most common being the Ellman method using the reagent DTNB. This method can be used to quantify -SH groups, but is not specific to glutathione. It is in this context that we have applied our methodology for GSH determination to yeast derivatives (YDs). The GSH and GSSG concentrations in 15 commercial YDs of 6 different brands were measured (Figure 2). The results show that both compounds were detected in all samples, with a mean value of 7.1 and 1.7 mg/g for GSH and GSSG respectively. Concentrations of GSH were significantly higher than those of GSSG, with values of up to 40 mg/g for GSH compared with 5.1 mg/g for GSSG (Figure 2). In general, the glutathione content of these commercial products is quite variable. Precise determination of GSH in YDs is thus essential to guide their use according to the winemaking techniques used and the target wine style.

Conclusion

This paper proposes an optimized method for the extraction and quantitation of glutathione potential in white wine lees and yeast derivatives. This methodology can be applied by oenological laboratories equipped with a liquid chromatography-mass spectrometry instrument. The extraction and quantitation methodologies may be of

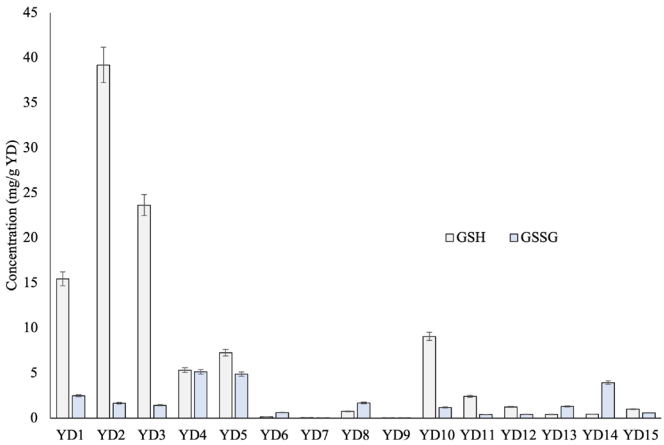


FIGURE 2. GSH and GSSG concentrations in 15 commercial yeast derivatives. Optimized extraction conditions: water/ethanol 50 % and YD at 10 g/L for 30 min.

interest for the determination of GSH and hence for more controlled management of aging on lees and the use of yeast derivatives in winemaking, with a view to conserving GSH levels. ■

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