

# Research Note: Total Free Sulphydryls of Several White and Red Wines

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**Total free sulphydryl groups were assessed in several white and red wines. Ellman's method was adapted to wine samples for the determination of total –SH groups. Total –SH groups of white wines, as glutathione, were in the range of 315 to 734 and of red wines in the range of 163 to 467 mg/L. In most cases, white wines exhibited higher values than red wines. The high total sulphydryls of white and red wines indicate their potential contribution to wine antioxidant capacity.**

## INTRODUCTION

Wines are rich pools of natural antioxidants. Phenolics are their major antioxidants, while amino acids and peptides containing –SH are also present. Wines contain several mg of the tripeptide glutathione, and also some amounts of other thiols such as cysteine and N-acetyl-cysteine. Glutathione retards must oxidation and possibly plays a role in wine browning. Moreover, glutathione and also N-acetyl-cysteine protect several wine aroma volatiles (Singleton *et al.*, 1985; Cheynier *et al.*, 1986; Vaimakis & Roussis, 1996; Park *et al.*, 2000; Papadopoulou & Roussis, 2001; Dubourdiou & Lavigne-Cruege, 2004; Roussis *et al.*, 2007; Papadopoulou & Roussis, 2008; Roussis & Sergianitis, 2008; El Horsy *et al.*, 2009; Roussis *et al.*, 2009; Kritzinger *et al.*, 2013). Some thiols play significant physiological roles in vivo as nucleophiles and scavengers of free radicals (Friedman, 1994, 1996). However, there are no reports about the potential impact of wine glutathione and other thiols on human health.

Amino acids and peptides containing free –SH in wines are determined by HPLC methods and also by capillary electrophoresis (Cheynier *et al.*, 1989; Lavigne *et al.*, 2007; Du Toit *et al.*, 2007). Moreover, a spectrophotometric procedure has been reported for the determination of total sulphydryls in red wines (Cimino *et al.*, 2007).

The aim of the present work was to determine the total free sulphydryl groups of white and red wines. To aid in this effort, Ellman's method for the assessment of sulphydryls was adapted.

## MATERIALS AND METHODS

DTNB (5,5'-dithio-bis (2-nitrobenzoic acid) reagent, glutathione and absolute ethanol were purchased from Sigma-Aldrich, while TPTZ, 2,4,6-Tri-2-pyridyl-1,3,5-triazine and (2R, 3R)-(+)-tartaric acid of 99.0% purity were purchased from Merck. A Consort C831 pH-meter,

an analytical balance Kern 770 and a Jenway 6505 UV/Vis spectrophotometer with glass or quartz cells of 1 cm were used.

Twenty-two dry wines from Greek vineyards were examined. Moschofilero, Assyrtiko, Moschato Alexandrias, Robola, Debina, Roditis, Agiorgitiko and Xinomavro wines were classed as "Appellation of Origin", while Vilana, Athiri, Savatiano, Malagousia, Trebbiano, Chardonnay, Traminer, Riesling, Sauvignon blanc, Cabernet Sauvignon, Kotsifali-Mandilaria blend, Limnio, Merlot and Syrah were regional wines. Most of the white wines were one year old, while Traminer and Riesling were two years old. Most red wines were two years old, while Xinomavro was three years old.

Total free sulphydryl groups (–SH) were determined using Ellman's reagent (Ellman, 1959; Bulaj *et al.*, 1998). In brief, 2.4 mL of phosphate buffer ( $K_2HPO_4/KH_2PO_4$ , 200 mM, pH 7.4) were mixed with 0.6 mL of wine sample (wine diluted 1:10 with model wine). Then, 0.3 mL of DTNB (5,5'-dithio-bis (2-nitrobenzoic acid) solution (1 mM in the same phosphate buffer) were added and the mixture was kept at 20°C for 1 h. For each sample, the absorbance at 412 nm was measured against a blank containing buffer instead of the DTNB solution. Moreover, the absorbance of a mixture consisting of 2.4 mL phosphate buffer, 0.6 mL model wine and 0.3 mL of the DTNB solution was subtracted from the sample absorbance. The spectrophotometer was set at zero using distilled  $H_2O$ . Results were expressed as glutathione equivalent. For this, glutathione solutions (0, 10, 20, 40, 80, 120 mg/L) in model wine were used instead of wine samples ( $C = 148 \times \text{Absorbance}$ ,  $R^2 = 0.9996$ ).

For the standardisation of conditions used in –SH determination, preliminary experiments were done using glutathione solutions in model wine and also wine. Using glutathione solutions, we used increasing concentrations

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of phosphate buffer with different pH values. Measured absorbances increased by increasing the concentration and/or pH values of the buffer used. Maximum absorbances were taken using 200 mM and pH 7.4. Similar results were also obtained to those using 1M phosphate buffer, pH 8.0 ( $C = 143 \times \text{absorbance}$  with an  $R^2 = 0.9986$ ). Moreover, we used 20 or 30°C for the reaction temperature and similar results were obtained. Similar results were also achieved using reaction times of one or two hours. We therefore chose phosphate buffer 200 mM, pH 7.4, and reaction conditions of one hour at 20°C. Finally, we applied the chosen conditions using different dilutions of a white (Roditis) and a red (Xinomavro) wine. For Roditis the equation was  $\text{Concentration} = 0.31 \times \text{Absorbance}$  with  $R^2 = 0.9978$ , and for Xinomavro it was  $\text{Concentration (fraction of wine in the unit of sample used)} = 0.47 \times \text{Absorbance}$  with  $R^2 = 0.9982$ . Wine concentration was defined as 1/dilution fold. After these preliminary experiments, we used the chosen conditions for the determination of total sulphydryls in 15 white and 7 red wines.

The model wine used consisted of 12% ethanol and 5 g/L tartaric acid in water, with the pH adjusted to 3.5 using 1N NaOH.

All analyses were done in triplicate and the results reported are the means along with the standard deviations.

## RESULTS AND DISCUSSION

We standardised a procedure for the determination of total free sulphydryls in wines using Ellman's reagent. Preliminary work had shown that the concentration and pH of the phosphate buffer used play a significant role, and that the chosen characteristics of the buffer (200 mM, pH 7.4) give maximum results. In Fig. 1, total free sulphydryls of different dilutions of a white and a red wine are presented. In both cases a linear equation was taken by plotting –SH values and wine dilution, indicating the suitability of the procedure.

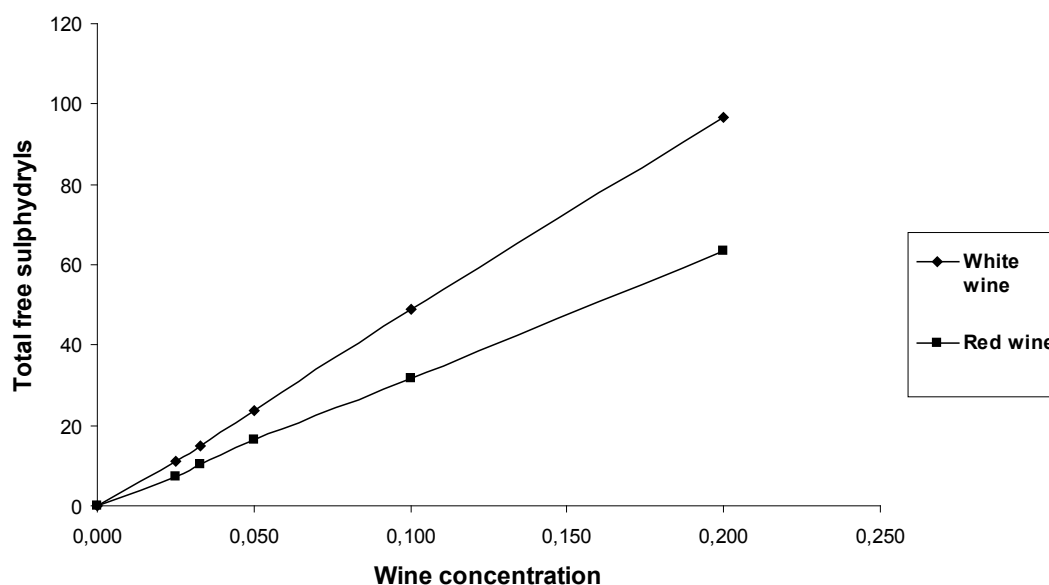


FIGURE 1

Total free sulphydryls of different concentrations (dilutions) of a white (Roditis) and a red (Xinomavro) wine. Total sulphydryl groups were expressed as glutathione equivalent in mg/L. Wine concentration was defined as 1/dilution fold.

Table 1 shows the total free sulphydryl groups of several white and red wines, expressed as glutathione equivalent in mg/L. In most cases, white wines exhibited higher total free sulphydryls than red wines. The range of 14 of the 15 white wines was 425 to 734 mg/L and of six of the seven red wines was 163 to 333 mg/L. One white wine (Riesling) exhibited a value (315 mg/L) in the range of red wines, and one red wine (Kotsifali-Mandilaria blend) had a value (467 mg/L) in the range of white wines. In another work, total free –SH groups in red wines were determined and the values reported were much lower than the values in the present work (Cimino *et al.*, 2007). However, this difference is due rather to the methodologies applied. It is possible that only part of total free –SH groups was determined in the previous work due to the low concentration of the phosphate buffer used.

The method used determines the total free sulphydryls, i.e. the sum of the total free sulphydryl groups of amino acids-small peptides and those of polypeptides-proteins. So, the higher values of white wines can be attributed to the removal of polypeptides-proteins from the red wines, after their reaction with tannins. Among wine thiol amino acid-small peptides, it is known that glutathione plays a significant role in must oxidation and wine aroma protection, and it exists in higher concentrations than the others (Vaimakis & Roussis, 1996; Papadopoulou & Roussis, 2001; Dubourdiou & Lavigne-Cruege, 2004; Du Toit *et al.*, 2007; Lavigne *et al.*, 2007; Roussis *et al.*, 2007; Papadopoulou & Roussis, 2008; Roussis & Sergianitis, 2008; Roussis *et al.*, 2009; Kritzinger *et al.*, 2013).

On the other hand, there are no reports on the potential antioxidant action of polypeptides-proteins containing free –SH. It has been shown that the antioxidant and aroma protection activities of some thiols in foods are due to their free sulphydryl group (Roussis *et al.*, 2009). It therefore could be expected that free sulphydryls of polypeptides-proteins contribute to wine antioxidant capacity.

TABLE 1  
Total sulphhydryl groups of several white and red wines.

White wines	Total -SH	Red wines	Total -SH
Moschofilero	425 ± 4	Agiorgitiko	239 ± 2
Assyrtiko	472 ± 4	Cabernet Sauvignon	163 ± 3
Vilana	435 ± 3	Kotsifali-Mandilaria blend	467 ± 1
Muscat of Alexandria	427 ± 1	Limnio	352 ± 3
Athiri	734 ± 4	Xinomavro	333 ± 7
Robola	573 ± 3	Merlot	185 ± 4
Debina	603 ± 2	Syrah	174 ± 1
Savatiano	502 ± 4	Mean/range	273/163-467
Malagousia	431 ± 2		
Trebbiano	622 ± 4		
Roditis	514 ± 2		
Chardonnay	645 ± 5		
Traminer	567 ± 4		
Riesling	315 ± 2		
Sauvignon blanc	436 ± 5		
Mean/range	513/315-734		

Total sulphhydryl groups (-SH) were expressed as glutathione equivalent in mg/L. Values are the means of analysis in triplicate along with standard deviation.

## CONCLUSIONS

The present results show that wines exhibit high concentrations of total free sulphhydryls, as determined by an appropriate adaptation of Ellman's method.

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