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# Must oxygenation together with glutathione addition in the oxidation of white wine

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White wines were made from oxygenated musts with or without glutathione addition and without $SO_2$. Must oxygenation led to wines with lower phenolic contents and browning capacity, and lower non-polymeric and polymeric phenolics in relation to control (made with $SO_2$ addition) wines. These wines were of good colour but their flavour was not typical of the grape variety used. The wines that were produced from oxygenated musts, together with glutathione addition, appeared to have somewhat higher total phenolics and browning capacity and non-polymeric and polymeric phenolics than those produced from oxygenated musts alone. Glutathione addition and must oxygenation led to wines of acceptable and stable colour with good flavour typical of the grape variety used.


## INTRODUCTION

The oxidation of white wines is a well-known problem in wine technology (Simpson, 1982; Singleton, 1987).

The removal of phenolic compounds by must oxygenation or enzymes is a promising method for controlling white wine oxidation (Cantarelli & Giovanelli, 1991; Guerzoni *et al.*, 1981; Vaimakis & Roussis, 1993).

Must oxygenation leads to wines with acceptable and stable colour. In addition, this 'natural' process can possibly be applied with little or no added $SO_2$. However, these wines lose their typical aroma, and the correct oxygen dose is open to question (Cantarelli & Giovanelli, 1991; Cheynier *et al.*, 1991; Guerzoni *et al.*, 1981; Maier *et al.*, 1990; Mueller-Spaeth, 1991; Nagel & Graber, 1988; Vaimakis & Roussis, 1993). Glutathione is a significant factor in must oxidation; it traps caftaric acid quinones, while it also acts as a general reducing agent (Cheynier *et al.*, 1986, 1990; Rosa & Maglitto, 1972; Singleton *et al.*, 1985).

This work explores the oxygenation of must together with glutathione addition in order (1) to study the changes in phenolic composition, and (2) to obtain white wines with acceptable and stable colour and typical flavour.

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## MATERIALS AND METHODS

### Must preparation

Grapes used were of the 'Debina' variety. 'Debina' is a late-ripening, easily oxidizable variety which is cultivated at Zitsa (Epirus, Greece).

Handmade preparations (destemming, crushing and juice extraction) were applied and so the free-run juice was used.

### Must vinification

In the first experimental section (first year) the must was divided into three portions, 2 litres each, which were treated as follows:

1a: addition of $SO_2$ 130 mg litre$^{-1}$.

1b: addition of $O_2$, 15 min (no $SO_2$ addition).

1c: addition of $O_2$, 15 min, and glutathione 0.2 g litre$^{-1}$ (no $SO_2$ addition).

The oxygen flow rate was 8 litres min$^{-1}$ and the temperature 18–20°C.

The must contained 185 g reducing sugars litre$^{-1}$, and the acidity was 7.4 g litre$^{-1}$ as tartaric acid. Total phenolics were 250 mg litre$^{-1}$ and catechins 100 mg litre$^{-1}$.

In the second experimental section (second year) the must was divided into four portions, 2 litres each, which were treated as follows:

2a: addition of $SO_2$ 130 mg litre$^{-1}$.

**Table 1. Characteristics of wines**

| Wine | Alcohol (ml per 100 ml) | Total acidity tartaric acid (g litre$^{-1}$) | Volatile acidity acetic acid (g litre$^{-1}$) | Total $SO_2$ (mg litre$^{-1}$) |
|---|---|---|---|---|
| 1a | 11.6 | 5.5 | 0.35 | 107 |
| 1b | 11.4 | 5.8 | 0.65 | – |
| 1c | 11.5 | 5.7 | 0.50 | – |
| 2a | 11.7 | 6.1 | 0.35 | 110 |
| 2b | 11.6 | 6.0 | 0.45 | – |
| 2c | 11.3 | 6.2 | 0.42 | – |
| 2d | 11.7 | 6.3 | 0.40 | – |

2b: addition of $O_2$, 10 min.

2c: addition of $O_2$, 10 min, and glutathione 0.2 g litre$^{-1}$.

2d: addition of $O_2$, 5 min, and glutathione 0.2 g litre$^{-1}$.

In the cases of 2b, 2c and 2d musts, $SO_2$ was not used. The oxygen flow rate was 8 litres min$^{-1}$ and the temperature 18–20°C.

The must contained 195 g reducing sugars litre$^{-1}$ and the acidity was 7.2 g litre$^{-1}$ as tartaric acid. Total phenolics were 220 mg litre$^{-1}$ and catechins 95 mg litre$^{-1}$.

In both sections the fermentation was carried out at 18–20°C and lasted 20–25 days. At the end of fermentation the wines were decanted, filtered and put into green glass bottles (0.75 litre). Bottles were stored at 15–20°C.

### Wine analysis

Alcohol, total acidity, volatile acidity, reducing sugars and total $SO_2$ were determined after the end of fermentation. The other wine parameters were measured 3 and/or 7 months after the start of fermentation.

Reducing sugars were determined by the Lane–Eynon method. Alcohol was determined pycnometrically, total acidity by volumetric analysis, volatile acidity by steam-distillation and total sulphite by the Ripper method.

Total phenolics were determined by the Folin–Ciocalteu method, and catechins and procyanidins were also determined spectrophotometrically (Amerine & Ough, 1980).

In the accelerated browning test, the method of Singleton & Kramling (1976) was followed.

Non-polymeric and polymeric phenolics were separated by using a Sephadex LH-20 column (Kantz & Singleton, 1990). Wine samples were evaporated and concentrated to 2.0–2.5 g total phenolics litre$^{-1}$ by rotary evaporator prior to loading onto the column. Void volumes of the chromatographic columns (diameter 1 cm, height 7 cm) were measured by running Blue Dextran. Non-polymeric phenolics were eluted by 15 void volumes of 60% methanol (0.2% acetic acid) and the following polymeric phenolics by 15 void volumes of 50% acetone (0.2% acetic acid). Flow rates were about 10 ml h$^{-1}$. Total phenolics in the two eluents were determined by the Folin–Ciocalteu method.

All experiments were run in duplicate and results reported here are the mean values of two runs.

## RESULTS AND DISCUSSION

All experimental wines were dry (< 2 g litre$^{-1}$ reducing sugars). The other characteristics of the wines produced are shown in Table 1. No differences were observed except for the higher values of volatile acidity of wines produced from oxygenated musts (1b wines). This is logical since oxygen promotes the growth of acetic acid bacteria. However, low must oxygenation led to wines with low volatile acidity, even without $SO_2$ addition (2b wines). Moreover, the addition of glutathione rather had a positive effect (1c and 2c wines), possibly acting as a reducing agent.

In Table 2 total phenolics, catechins and procyanidins of 3- and 7-month wines are presented. It can be seen that must oxygenation led to removal of phenolics, especially procyanidins. Losses in all procyanidins by hyperoxidation (possibly oxidized by enzymically generated quinones) have also been reported by others (Cheynier & Silva, 1991; Silva *et al.*, 1993). On the other hand, the addition of glutathione decreased the removal of phenolics observed by must oxygenation.

The wines produced from oxygenated musts developed lower values in the browning capacity test than control wines (Table 3). The low content of phenolics, together with the low browning capacities of these wines, indicate that they had been subjected to oxidation (browning) to a greater extent, since the oxidation products are precipitated (Singleton & Kramling, 1976; Singleton, 1987). Glutathione addition led to some increase of browing susceptibility of wines produced

**Table 2. Total phenolics, catechins and procyanidins of 3- and 7-month wines**

| Wines | Total phenolics (mg litre$^{-1}$ gallic acid) | | Catechins (mg litre$^{-1}$ (+)-catechin) | | Procyanidins (mg litre$^{-1}$) | |
|---|---|---|---|---|---|---|
| | 3 months | 7 months | 3 months | 7 months | 3 months | 7 months |
| 1a | 150 | 150 | 13 | 6 | 60 | 54 |
| 1b | 100 | 100 | 8 | 6 | 9 | 13 |
| 1c | 110 | 112 | 8 | 4 | 22 | 18 |
| 2a | 170 | 160 | 20 | 15 | 53 | 45 |
| 2b | 110 | 105 | 8 | 8 | 10 | 7 |
| 2c | 130 | 120 | 8 | 7 | 15 | 10 |
| 2d | 150 | 140 | 13 | 10 | 25 | 20 |

**Table 3. Browning capacity test of 7-month wines**

| Wine | Absorbance at 420 nm | | |
|---|---|---|---|
| | Treatment with oxygen | Treatment with nitrogen | Difference |
| 1a | 0.456 | 0.101 | 0.355 |
| 1b | 0.200 | 0.077 | 0.123 |
| 1c | 0.336 | 0.093 | 0.249 |
| 2a | 0.430 | 0.100 | 0.330 |
| 2b | 0.210 | 0.075 | 0.135 |
| 2c | 0.250 | 0.085 | 0.165 |
| 2d | 0.330 | 0.090 | 0.240 |

from oxygenated musts. Glutathione traps quinones produced during must oxidation (Cheynier *et al.*, 1986, 1990; Singleton *et al.*, 1985). Thus it is logical that the addition of glutathione leads to higher phenolic contents and subsequently to higher browning capacities.

In Table 4 non-polymeric phenolics of experimental wines 2a–d are presented. It can be seen that must oxygenation led to removal of both non-polymeric and polymeric phenolics (2b wines). This is logical since the oxidation of phenolics is followed by the precipitation of the polymeric oxidation products. On the other hand, the addition of glutathione decreased the above removal of non-polymeric and polymeric phenolics (2c wines), possibly by trapping generated quinones.

All wines produced exhibited a pale yellow colour with a green hue.

Control wines (1a and 2a wines) were typical of the grape variety used, with fruitiness and freshness. Wines produced from high-oxygenated musts (1b wines) presented an oxidation flavour and a lack of fruitiness, which has also been observed by others (Singleton *et al.*, 1979). On the other hand, wines produced from high-oxygenated musts together with glutathione addition (1c wines) were much better, without the distinct oxidation flavour.

Wines produced from intermediate-oxygenated musts (2b wines) presented a simple flavour without freshness, although they were ranked as wines of good quality. However, wines produced from intermediate-oxygenated musts toghether with glutathione addition (2c wines) presented a complex fruity aroma, a balanced and fresh taste and an aromatic after-taste. These wines exhibited all the aromatic characters of the grape variety used. Recovery of varietal aroma by glutathione addition in wines has also been reported by others (Doubourdieu & Darriet, 1993). On the other hand, wines produced from low-oxygenated musts, together with glutathione addition (2d wines), developed a complex aroma and a balanced taste but an after-taste with heavy flavour.

## CONCLUSIONS

Must oxygenation leads to white wines of low phenolic content with nice colour and low browning capacity. On the other hand, wines that are produced by this process lose their aromatic characteristics and fruitiness. As must oxygenation increases, the magnitude of the above effects increase too.

**Table 4. Non-polymeric and polymeric phenolics in 7-month wines**

| Wine | mg per 15 mg total phenolics[a] | | mg litre$^{-1}$ wine[b] | |
|---|---|---|---|---|
| | Non-polymeric phenolics | Polymeric phenolics | Non-polymeric penolics | Polymeric phenolics |
| 2a | 14.1 | 0.9 | 150 | 9.6 |
| 2b | 14.4 | 0.6 | 101 | 4.2 |
| 2c | 14.6 | 0.7 | 117 | 5.6 |
| 2d | 14.2 | 0.8 | 133 | 7.5 |

[a]Results obtained from the run of samples through the Sephadex LH-20 column.
[b]Results obtained by calculating the Sephadex LH-20 column results and the total phenolic content of wines.

Results of the present work indicate that the addition of glutathione together with must oxygenation can lead to white wines of acceptable and stable colour and typical of the grape variety that is used. However, the correct oxygen dose and amount of glutathione should be determined for the desired result.

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