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Level of single bioactive phenolics in red wine as a function of the oxygen supplied during storage

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Abstract

The influence of three levels of oxygen supply on the phenolic composition of Sangiovese wine was evaluated during 6 months of storage. Oxygenation reduced the total phenolics content and increased the concentration of red polymeric compounds. Saturation with oxygen every 30 days significantly improved the wine colour density as compared to the control. The individual behaviour of phenolic compounds such as (+) catechin, (−) epicatechin, quercetin, caffeic acid, and anthocyanins showed a significant depleting effect of oxygen on wine phenolic composition when wines were oxygenated every month. The supply of oxygen every 60 days limited the oxidation of low molecular weight phenolic compounds and slightly influenced the wine colour. This confirmed that oxygenation could improve the evolution of red wines during ageing, but its control is necessary to reduce detrimental effects upon single bioactive phenolic compounds.

1. Introduction

Many studies have underlined that phenolics in grapes and wines may act as antioxidants in vitro, limiting the free-radical damage due to reactive oxygen species (Frankel, Kanner, German, Parks & Kinsella, 1993; Kanner, Frankel, Granit, German & Kinsella, 1994; Vinson & Hontz, 1995). On the other hand, the health benefits associated with dietary phenols are not completely proved, because their absorption and effectiveness in enhancing protective mechanism are largely unknown in vivo. Nevertheless, some recent studies seem to confirm that the ingestion of natural antioxidant as wine phenols is consistent with higher protection against oxidation in vivo (Durak, Koseoglu, Kacmaz, Buyukkocak, Cimen & Osturk, 1999; Harada et al., 1999; Koga et al., 1999; Ray, Maulik, Cordis, Bertelli, Bertelli & Das, 1999; Roig, Cascon, Arola, Blade & Salvado, 1999; Sanderson, McLauchlan & Williamson, 1999; Sato, Maulik, Ray, Bagchi & Das, 1999; Wollny et al., 1999).

In particular red wines were indicated as the most potent alcoholic beverage conferring protection against atherosclerosis and thrombotic diseases (German, Frankel, Waterhouse, Hansen & Walzmen, 1997; Renaud, Beswick, Fehily, Sharp & Elwood, 1992). It has also been stated that total antioxidant activity (or the radical scavenger activity) of wines is well correlated with the total phenolics content (Sato, Ramarathnam, Suzuki, Ohkubo, Takeuchi & Ochi, 1996; Vivas, Saint-Cricq de Gaulejac & Glories, 1997).

Anthocyanins, gallic acid, (+) catechin, quercetin, caffeic acid, (−) epicatechin and resveratrol, are at least twice as active as α-tocopherol (Lapidot, Harel, Akiri, Granit & Kanner, 1999; Meyer, Heinonen & Frankel, 1998; Mili'c, Djilas & Canadanovi'c-Brunet, 1998). On the basis of such evidence, Soleas, Tomlinson, Diamandis and Goldberg (1997) proposed a predictive model to evaluate the total antioxidant activity of wines, based upon single phenolic compound concentrations. On the other hand, the content of total and single phenolics in red wines can be strongly influenced by many variables, depending both as grape composition and enological practices connected with the winemaking process (Castellari, Arfelli, Riponi & Amati, 1998; Castellari, Spinabelli, Riponi & Amati, 1998; Dallas & Laureano, 1994; Mattivi, Reniero & Korhammer, 1995; Pezet & Cuenat, 1996). Moreover, red wines are often aged in stainless steel tanks or oak barrels. During this phase, many reactions can lead to the formation of polymeric

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pigments by direct condensation of anthocyanins with other flavonoids or a combination of pigments with acetaldehyde. These reactions cause a modification of phenolics with the production of highly coloured compounds, and reduce astringency, affecting both the wine colour and the palatability (Bakker, Picinelli & Bridle, 1993; Bishop & Nagel, 1984; Somers & Evans, 1986).

The rate of the progressive decline in anthocyanins and the formation of new, stable colour pigments is influenced by such factors as temperature, pH, sulphites and oxygen content. The capacity of a red wine to take up oxygen ranges from several to many times the saturation level — 8 mg/l or 6 ml/l- (Singleton, 1987).

Red wines display a wide range of responses, but generally improve at least up to about 60 ml/l and begin to be lowered in quality beyond 150 ml/l (Boulton, Singleton, Bisson & Kunkee, 1996). A mild condition of oxygenation was proposed for amelioration of wine quality during storage both in stainless steel tanks and oak barrels (Moutonet, Ducournau, Chassin & Lemaire, 1996; Pontallier & Ribéreau-Gayon, 1983). In spite of the different behaviour of low molecular weight phenolic compounds upon oxidation (Barroso, Palma & Perez-Bustamante, 1995), the quantity of oxygen supplied during red wine ageing is generally not precisely measured or controlled and the effects of controlled oxygenation and different oxidation status on the level of the single bioactive phenolic compounds are therefore, not well known.

The aim of this work was to evaluate the influence of controlled levels of oxygenation on red wine colour and composition during storage, emphasising its effect on the concentration of some low molecular weight phenolic compounds, well known for their physiological activity.

2. Materials and methods

2.1. Musts and wines

A batch of 1300 kg of red Sangiovese grapes was destemmed, crushed, treated with 150 mg/l of $K_2S_2O_5$ and placed in a vertical 1500 l stainless steel tank. The must was then inoculated with active dry wine yeast (*Saccharomyces cerevisiae* 404 IMIA — contained in DI.PRO.V.AL, University of Bologna strain collection) and the maceration was conducted at 25°C for 7 days. Both pressed and free-run juices were assembled and fermented to dryness in a 1000 l stainless steel tank. The wine was then racked and filtered through a membrane filter (1 μm). $K_2S_2O_5$ was introduced until 30 mg/l of total sulphites was reached and then the wines were saturated with N_2. Within a few days the red wine was filled (by using N_2) into twelve 50 l stainless steel tanks and stored in a cellar at 15°C for 6 months. During wine conservation, three levels of oxygen were supplied. In four tanks, no oxygen was added (control). Four other tanks, were supplied every 2 months with pure oxygen with saturation level (8 mg/l) was reached (Oxy-A). The final four tanks were dispensed monthly with pure oxygen up to saturation (Oxy-B). Oxygen (1 bar — flow rate of 10 ml/min) was supplied through a microdiffuser, recirculating the wine of each single tank with a peristaltic pump at a flow of 500 ml/min.

During the maturation phase, 50 ml of wine were taken (under N_2) from each tank after 60, 120 and 180 days, cooled by using carbonic ice and immediately evaluated for colour and total phenolic content. After 6 months of storage, samples of wine were taken under the same conditions and analysed as described below.

2.2. Analytical procedures

Dissolved oxygen was measured every 30 days, always before each oxygen saturation and without any air contact. Measurements were taken electrochemically by recycling the wines with a peristaltic pump through an Oxytech model 26073 (Orbisphere Corporation, Switzerland).

Ethanol, pH, total sulphites, malic and L(-) lactic acids, colour parameters and total phenolics were measured according to the Official Methods (ECC, 1990). Proanthocyanidins were evaluated by a reaction based upon their transformation into anthocyanins when heated in an acidic medium (Ribèreau-Gayon, Pontallier & Glories, 1983). Dialysis index was measured according to Ribèreau-Gayon et al., using a membrane dialysis with a nominal molecular weight (MW) cut-off of 3500 (Cellu-Sep T1, Membrane Filtration Products Inc., San Antonio- US) immersed in synthetic wine for 72 h at 20°C. The polymeric fraction (phenolics with $MW > 3500$) was calculated as a percentage of total phenolics. All spectrophotometric determinations were made using a Uvidec 430 (Jasco Co., Tokyo- Japan).

2.3. HPLC analyses

Anthocyanin separation was carried out by the HPLC method according to Castellari, Arfelli et al.(1998), with a Knauer liquid chromatograph equipped with a RP Hypersil C_{18} (Phenomenex, Torrance, US) column (5 μm packing, 4.6 mm id×150 mm). The elution programme involved mixtures of 6% aqueous perchloric acid and methanol at a flow rate of 1 ml/min. Samples were diluted in the mobile phase, filtered through a 0.45 μm PTFE membrane filter and injected (20 μl loop).

Other phenolic compounds, such as phenolic acids, flavonoids and resveratrol were evaluated by HPLC, with a method slightly modified from Castellari, Karakaidos, Arfelli, Amati and Torsi (1998). Analyses were carried out with a Jasco 880-PU pump equipped with an 880-02 ternary gradient unit and a MD-910 Diode Array Detector (Jasco Co., Tokyo, Japan). Separation

was carried out with an Inertsil ODS-2 (GL Science Inc., Japan) column (5 μm packing, 4.0 mm id×250 mm) maintained at 35°C using a column heater 7980 (Jones Chromatography Ltd., Hengeod, UK). Eluent A (MeOH: double-distilled water 5:95 at pH 3 with H_3PO_4) and eluent B (MeOH: double-distilled water, 50:50, at pH 3 with H_3PO_4) were freshly prepared and filtered (0.22 μm) before use. A linear gradient elution was performed at a flow of 0.5 ml/min as follows: 0–85 min, from 3% B to 70% B, then 100% B at 110 min. Samples were properly diluted in the mobile phase A, filtered through a 0.45 μm PTFE membrane filter and directly injected (10 μL loop). Detection was made at 280 nm for (+) catechin, (−) epicatechin, gallic acid and *cis*-resveratrol; at 308 nm for *trans*-resveratrol; at 324 nm for caffeic, ferulic, *trans*-caffeoyl-(+)-tartaric (caftaric) and *trans-p*-coumaroyl-(+)-tartaric (coutaric) acids; at 365 nm for quercetin. Fig. 1 shows the chromatographic profile of a wine sample. Compound identification was achieved by comparing the retention time and the spectra with those of pure standards (FLUKA Chemie AG-Switzerland, except *trans*-resveratrol furnished by SIGMA Chemical Company, St. Louis-USA). *Cis*-resveratrol was obtained by exposing the *trans* isomer to sunlight (Lamuela-Raventós, Romero Pérez, Waterhouse & de la Torre-Boronat, 1995). Caftaric acid and

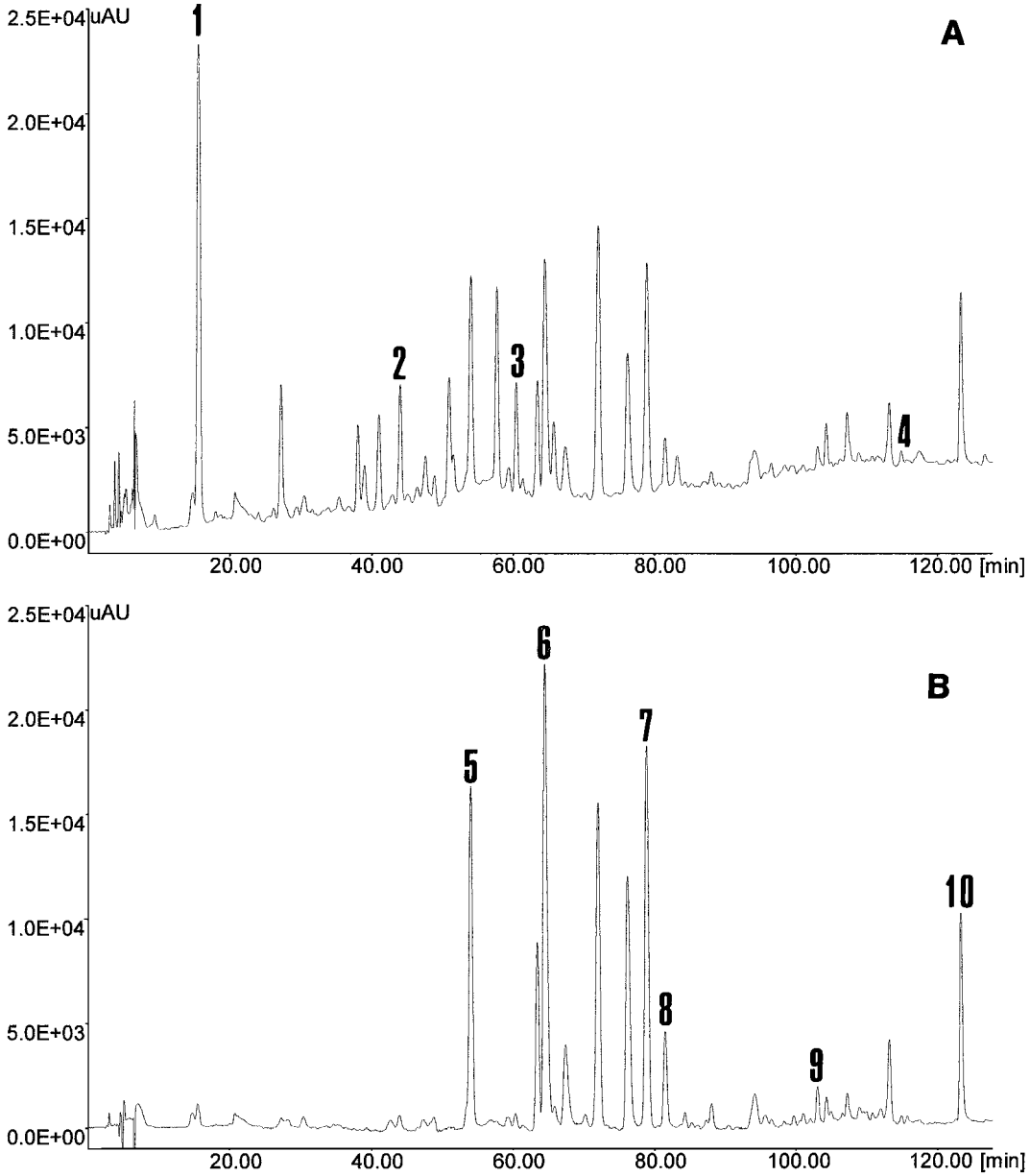

Fig. 1. HPLC chromatogram of phenolics separation in a red Sangiovese wine. (A) acquisition at 280 nm; (B) acquisition at 320 nm. (1) gallic acid, (2) (+) catechin, (3) (−) epicatechin, (4) *cis*-resveratrol, (5) caffeic acid, (6) caftaric acid, (7) ferulic acid, (8) coutaric acid, (9) *trans*-resveratrol, (10) quercetin.

coutaric acid were tentatively identified by comparing retention times and UV spectra with those reported in the literature and quantified as caffeic acid and *p*-coumaric acid, respectively. All analyses were done in duplicate.

2.4. Statistics

Analytical results were submitted to ANOVA and Tukey test to evaluate differences between means using Statistica (StatSoft, Inc., Tulsa, OK, US); different treatments were considered as independent variables and significance level was set at $P < 0.05$.

3. Results and discussion

After 6 months of maturation, malic and L(-) lactic acid contents indicated that malolactic fermentation did not take place in any sample. The ethanol, total sulphites, and pH were not significantly affected in the oxygenated wines as compared to the controls (Table 1). Dissolved oxygen in control wines quickly decreased during storage and, after 60 days, the oxygen concentration was close to the instrumental detection limit (Table 2).

In the Oxy-A and Oxy-B wines, oxygen consumption was at first rapid. Starting from the second saturation, the dissolved oxygen still present before every oxygenation increased, in agreement with the assumption that red wine's capacity to combine oxygen progressively decreases (Singleton, 1987).

The more oxygenated wines (Oxy-B) developed a more intense absorbance at 420 and 520 nm; while the control wines showed a decrease in red colour and an increase in brown colour (Fig. 2). The Oxy-A wines had a partial stabilisation of red colour but, at the end of the experimental period, their colour was very similar to that of the control wines. It is noteworthy that during the first 2 months of storage the oxygenation had a slight effect on colour. This effect became more pronounced at the end of storage, highlighting the essential role of oxygen in improving red wine colour during the maturation phase, in accordance with other authors (Pontallier & Ribéreau-Gayon, 1983).

At the end of storage, the Oxy-B wines had a higher colour density than the controls, while the colour hue was not significantly different. Oxy-A wines showed the

Table 2
Evolution of dissolved oxygen during red wine storage[a]

Days of storage	Dissolved oxygen (mg/l)		
	Control	Oxy-A[b]	Oxy-B[b]
0	0.8 (±0.1)	0.8 (±0.1)	0.8 (±0.1)
30	0.5 (±0.1)	1.0 (±0.2)	1.0 (±0.1)
60	0.2 (±0.1)	0.2 (±0.1)	2.9 (±0.4)
90	0.2 (±0.1)	2.8 (±0.4)	3.6 (±0.3)
120	0.2 (±0.1)	1.5 (±0.2)	3.8 (±0.4)
150	0.2 (±0.1)	4.0 (±0.5)	4.0 (±0.4)
180	0.2 (±0.1)	3.4 (±0.3)	4.2 (±0.5)

[a] Values are the average of four replicates (in brackets the S.D.).
[b] For Oxy-A and Oxy-B samples all measures were made before oxygenation.

Table 1
Wine composition after 6 months of storage[a]

		Control	Oxy-A	Oxy-B
Ethanol	%	12.8 n.s.	12.9 n.s.	12.9 n.s.
pH	20°C	3.35 n.s.	3.31 n.s.	3.34 n.s.
Total sulphites	mg/l	28.1 n.s.	27.5 n.s.	26.8 n.s.
Malic acid	g/l	1.98 n.s.	2.05 n.s.	1.96 n.s.
L(-)lactic acid	mg/l	36 n.s.	37 n.s.	38 n.s.
Colour density	Abs	6.313 a	6.363 a	6.918 b
Colour hue	Abs	0.682 n.s.	0.675 n.s.	0.678 n.s.
Total phenolics	mg/l	1621 c	1540 b	1488 a
Proanthocyanidins	mg/l	1217 b	1168 ab	1122 a
Dialisys index	%	35.5 a	42.6 b	49.0 c
Caftaric acid	mg/l	21.2 n.s.	21.0 n.s.	21.3 n.s.
Coutaric acid	mg/l	13.0 n.s.	12.9 n.s.	13.1 n.s.
Gallic acid	mg/l	15.9 b	15.3 ab	15.1 a
Caffeic acid	mg/l	5.2 b	5.0 b	4.0 a
Ferulic acid	mg/l	2.1 b	1.9 b	1.4 a
(+) Catechin	mg/l	32.8 b	32.0 ab	30.3 a
(−) Epicatechin	mg/l	16.1 b	15.2 ab	15.1 a
Quercetin	mg/l	3.5 c	2.7 b	1.4 a
trans-resveratrol	mg/l	0.535 b	0.520 ab	0.500 a
cis-resveratrol	mg/l	0.107 n.s.	0.100 n.s.	0.100 n.s.

[a] Letters on the same lines indicate means separation at $P < 0.05$.

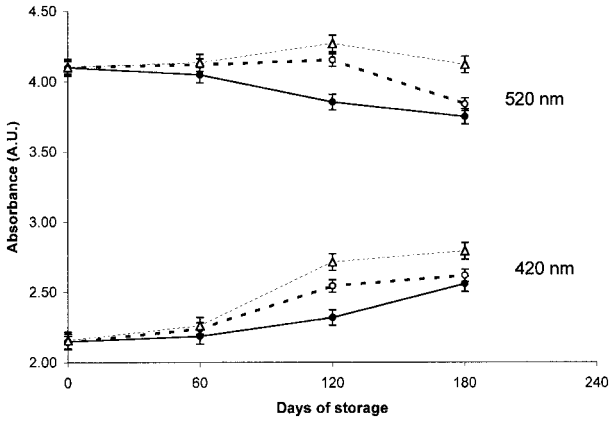

Fig. 2. Evolution of red (520 nm) and brown (420 nm) colour in oxygenated and control wines during storage. ——● Control wines; - - - -○ Oxy-A wines; ·····△ Oxy-B wines.

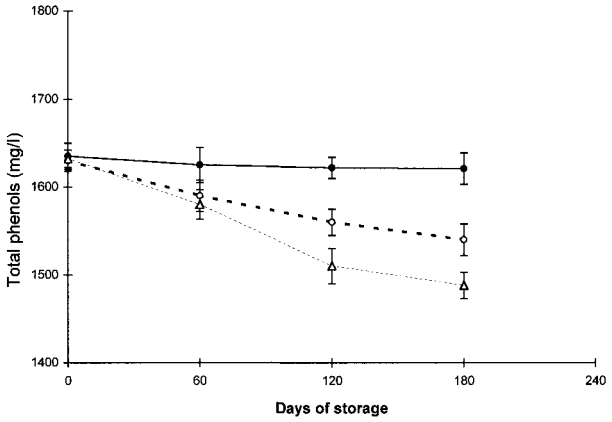

Fig. 3. Evolution of total phenolics in oxygenated and control wines during storage. ——● Control wines; - - - -○ Oxy-A wines; ·····△ Oxy-B wines.

same colour characteristics of the control wines (Table 1). The total phenolic content progressively decreased in oxygenated wines as compared to the control wines in which this parameter remained constant (Fig. 3). Oxygenation caused a significant reduction of total phenolic content after 180 days of storage (Table 1). Proanthocyanidins were affected only by the more intense oxygen treatment (Oxy-B). The amount of all single anthocyanins was reduced in oxygenated wines (Fig. 4). In Oxy-B wines, the average reduction was about 50% as compared to the controls. In Oxy-A wines, the behaviour of delphinidin-3-glucoside and cyanidin-3-glucoside was an exception, since their concentrations were not significantly different from the controls. The percentage of polymeric phenols (dialysis index) showed that oxygenated wines had a significantly higher content of large molecular weight phenolics (Table 1). Reactions involved in the progressive formation of red polymeric pigments during maturation of red wines seem to be essentially anaerobic (Somers & Evans, 1986). Our results, however, according to the above mentioned increase of red colour in oxygenated wines as compared to the control wines kept in an anaerobic condition, indicate that oxygen significantly influenced the formation of red pigmented polymers and stabilised the red wine colour as stated in other studies (Bakker et al., 1993; Francia-Aricha, Guerra, Rivas-Gonzalo & Santos-Buelga, 1997; Singleton, 1987). Moreover, a considerable number of polyphenol transformation products have been identified as minor components of red wines and have been characterised in model systems (Fulcrand, Cameira-dos-Santos, Sarni-Manchado, Cheynier & Favre-Bonvin, 1996; Fulcrand, Cheynier, Oszmianski & Moutounet, 1997). Further specific studies could elucidate if and how elevated oxygen tension can influence the evolution of these compounds and their technological/sensorial interest.

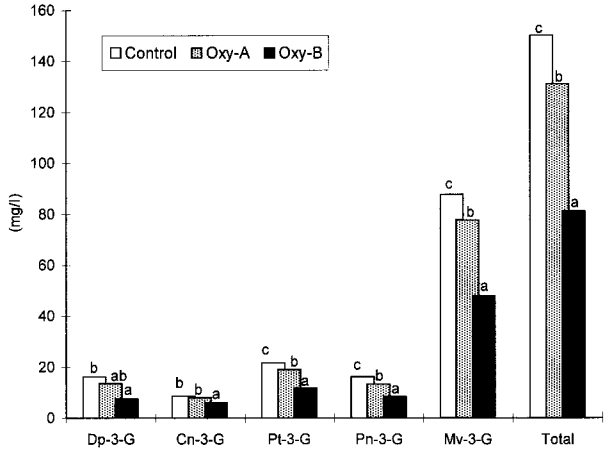

Fig. 4. Concentration of single anthocyanins in control and oxygenated wines after 6 months of storage. Dp-3-G = delphinidin-3-glucoside; Cn-3-G = cyanidin-3-glucoside; Pt-3-G = petunidin-3-glucoside; Pn-3-G = peonidin-3-glucoside; Mv-3-G = malvidin-3-glucoside; Total = sum of single anthocyanins. Small letters indicate a means separation at $P < 0.05$.

The oxygen dissolution, by enhancing the condensation and polymerisation reactions, could also influence the ability of phenolic compounds to act as antioxidants. As Baldi, Romani, Mulinacci, Vincieri and Ghiselli (1997) reported, condensation products, such as tannin-anthocyan (T-A) and tannin-anthocyan-tannin (T-A-T) showed a lower contribution to the antioxidant activity of wine than their monomeric compounds. Moreover, these authors stated that antioxidant activity of polymeric compounds decreases with the increase of their molecular weight.

Our study of individual behaviour of phenolic compounds indicated that caftaric and coutaric acid contents were not influenced by oxygen supplied to the wines (Table 1), even if they are mainly involved in phenolic enzymatic oxidation in grape musts (Singleton,

Table 3
Significant relationship (r^2) between analytical parameters ($n = 12$, $P < 0.05$)

	Colour density	Proanthocyanidins	Dialysis index	Total anthocyanins	Ferulic acid	(−) epicatechin	Quercetin
Total phenolics		0.84	−0.88	0.80			0.84
Proanthocyanidins	−0.83			0.92			
Dialysis index	0.83	−0.96		−0.92			
Total anthocyanins	−0.91						
Caffeic acid	−0.74		−0.74				0.71
Ferulic acid	−0.91	0.88	−0.85	0.95			
(+) Catechin	−0.75		−0.63	0.70	0.72	0.77	
(−) Epicatechin	−0.77		−0.71	0.77	0.82		0.71
Quercetin	−0.90	0.96	−0.96	0.97	0.94		
trans-resveratrol	−0.89	0.88	−0.87	0.93	0.94	0.85	0.91

1987). Low molecular weight phenolic compounds that exhibited a pronounced concentration reduction were gallic, caffeic and ferulic acid, (+) catechin, (−) epicatechin and *trans*-resveratrol. It is noteworthy that this significant phenolic depletion happened only in monthly oxygenated wines (Oxy-B).

The quercetin, on the contrary, was always influenced by oxygenation, indicating its higher reactivity with oxygen as compared to the other compounds.

Nevertheless, the total decrease of low molecular weight phenolic compounds was about 11% in the wines oxygenated every month compared to the control wines.

Considering that all of the wines were stored at the same temperature and with the same total sulphites level, oxygenation significantly affected the phenolic composition of red wine as showed for other important factors such as temperature and sulphites (Dallas, Ricardo Da Silva & Laureano, 1995).

The relationships between variables confirmed that colour density and the presence of polymeric phenolics (as stated by dialysis index) were directly related (Table 3). On the other hand, colour density and the dialysis index were inversely correlated with the concentration of all the single phenolic compounds and with the total anthocyanins. The content of the single phenolic compounds was also correlated with those of ferulic acid, total anthocyanins, quercetin and (−) epicatechin.

4. Conclusions

Adding oxygen to red wine during the maturation phase reduced total phenolic compounds and increased the red polymeric pigments with a stabilising effect on colour. A saturation with oxygen every 30 days significantly increased the red wine colour, confirming that oxygen could improve the evolution of red wines during ageing.

In spite of this positive effect, our results showed that the immoderate uptake of oxygen plays a detrimental role in wine quality, by significantly decreasing the amount of some monomer and oligomeric phenolic compounds known to be related to the antioxidant status of wine.

Therefore, on the basis of therapeutic studies, the harmful "secondary" effect of oxygen absorption on red wine properties indicates the need for a careful study of oxygen dissolution during red wine maturation. The effects of precise and controlled oxygenation on the degradation of phenolic compounds and their subsequent chemical evolution need to be better elucidated, since oxygen, like temperature and sulphites, is unquestionably an important technological factor in red wine maturation. So, from a practical point of view, it would be interesting to better manage the red wine maturation phase, controlling the exposure of red wine to oxygen, and exploring compositional, sensorial and commercial effects.

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