

Browning rate of white wines: Dependence on antioxidant activity kinetics and changes in phenolic composition

Kotseridis, Y^a., Kallithraka, S^a., Kyraleou, M.^a, Proxenia, N^a, Makris, D.P.^b

a : Department of Food Science & Technology, Agricultural University of Athens, 75 Iera Odos, 11855 Athens, Greece

b: Department of Food Science & Nutrition, University of the Aegean, Limnos, Greece

ABSTRACT

Browning is a serious problem in relation to white wine quality and mostly affects the sensory attributes, whereas its impact on the dietary value of wines has never been investigated. Browning, however, is well known to be associated with polyphenol oxidation, and therefore it may be accompanied by changes in the antioxidant capacity. The browning capacity and the antioxidant activity of eight white wines produced in Paros and Santorini islands were studied, employing an accelerated test. Browning was approached from a kinetic point of view and efforts were focussed on the investigation of plausible correlations with major redox-active polyphenols, including substances with an o-diphenol feature, such as catechin, and epicatechin. Regression analysis between k values and concentration of individual phenolics provided strong evidence that the depletion of (-)-epicatechin due to oxidation is the principal browning reason. Furthermore, the monitoring of the antioxidant activity, throughout treatments, indicated that increases in browning are accompanied by an increase in the antioxidant activity, providing evidence about the beneficial impact of browning reactions on the in vitro antioxidant properties of white wines.

Keywords: browning rate, antioxidant activity, wine, polyphenols

INTRODUCTION

Browning in wines is the result of a complex series of oxidation reactions that take place during processing, ageing and storage, which give rise to a brown color that increases color intensity, decreases brightness and raises the browning index (Singleton & Kramling, 1976; Gonzales Cartagena et al., 1994). These reactions may be enzymatic (Nagel & Graber, 1988) or non-enzymatic (Cilliers & Singleton, 1990). Non-enzymatic oxidation may occur in the absence of active polyphenol oxidase, resulting in the appearance of a more or less intense brown color and 'woody' aroma. This phenomenon is considered desirable in dessert wines but undesirable in young table wines, sparkling wines and mature red wines. The production of white wines involves a great effort to avoid extensive contact with oxygen, which might be deleterious in terms of color alteration (browning) and eventually deterioration of the overall quality and marketability.

The most important polyphenolic constituents in white wines, both in terms of quantity and ability to participate in redox reactions, are the hydroxycinnamates and flavanols. In particular, oxidation of *ortho*-dihydroxyphenolic compounds such as (+)-catechin, (-)-epicatechin, caffeic and other hydroxycinnamic acids leads to the formation of yellow or brown products due to the polymerization of *ortho*-quinones (Guyot, Vercauteren, & Cheynier, 1996). Other constituents of the wine such as transition metal ions and the presence of SO₂ and ascorbic acid are of equal importance in polyphenol oxidation (Singleton, 1987). Sulphur dioxide and ascorbic acid added to wine are able to reduce the *ortho*-quinones, while metal ions can catalyse oxidation reactions (Singleton, 1987).

Browning development in white wines is of both technological and nutritional significance due to its influence on wine organoleptic characters and antioxidant status. During storage, oxidation of principal polyphenolic compounds would presumably afford changes in the wine antioxidant status, as a consequence of changes in the redox equilibrium. Normally, one should expect oxidation of antioxidants to yield lower antioxidant capacity, but because reactions between oxidized phenolics may bring about formation of novel antioxidants, it would appear rather impossible to predict the antioxidant properties of wines having developed browning. On the basis of this concept, the issue concerned with browning in white wines should be addressed as being essential not only because of its influence on the organoleptic characters, but also owing to its importance pertaining to the antioxidant potential. Antioxidant activity is the most studied property in relation to the health benefits of wine consumption, and it has been related initially to the presence of flavonoids since they could function as both free radical terminators and metal chelators (Benítez, Castro, Sánchez Pazo & Barroso, 2002).

The present study was undertaken to examine which parameters are involved in browning development in certain white wines produced by native varieties grown in Greek islands and to evaluate browning rate in relation to its result on antiradical parameters and flavanol composition.

MATERIALS AND METHODS

Wines: Eight white wines were assayed, which were vinified in 2011. The cultivars used (Table 1) were *V. vinifera* species. The total SO₂ level was set to 100 mg/L prior to bottling. Some compositional factors of the wines examined are also given in Table 1.

Accelerated browning test

The model used to assess browning development (Sioumis, Kallithraka, Makris & Kefalas, 2006) was a modification of that described by Singleton and Kramling (1976). Wine lots of 20 ml were filtered through pharmaceutical cotton and placed in a 30-ml, screw-cap glass vial (7.5 cm length, 2.1 cm internal diameter). Samples were subjected to heating at a constant temperature of 55.0 ± 0.2 °C in a water bath, in obscurity. Each sample was divided into 12 glass vials, one for each day of analysis. Thus, in the water bath were placed in total 8 x 12 x 3 glass vials. One out of the 12 glass vials of each sample was withdrawn at 24-h intervals over a period of twelve days, and browning (A_{420}) was measured in triplicate against 12% ethanol.

Determination of total phenols (TP)

Total polyphenol concentration was determined with the Folin-Ciocalteu assay, with the micro scale protocol previously reported (Arnous, Makris & Kefalas, 2001). Results were expressed as mg l^{-1} gallic acid equivalents (GAE).

Determination of antiradical activity (A_R)

For the determination of the antiradical activity, assays were performed at 24-h intervals over the period of 12 days employing DPPH \cdot stable radical (Psarra, Makris, Kallithraka & Kefalas, (2002). Results were expressed as Trolox equivalents (mM TRE).

Determination of the analytical polyphenolic composition

The individual polyphenolic constituents were determined by HPLC at the beginning and at the end of the accelerated browning test. The chromatography apparatus used was an HP 1090, coupled with an Agilent 1100 diode array detector, and controlled

by Agilent ChemStation software. The column was a Spherisorb ODS 2 (AnalyzenTechnik, MZ, Germany), 250 x 4 mm, 5 μ m, protected by a guard column packed with the same material. Both columns were thermostatically controlled at 20 °C. The chromatographic conditions, as well as detection, identification and quantification of peaks were carried out as described previously (Makris, Psarra, Kallithraka, & Kefalas, 2003).

Statistical analysis

All determinations were run in triplicate and values were averaged. The standard deviation (S.D.) was also calculated. The percentage change in browning ($\% \Delta A_{420}$) was calculated as follows: $\% \Delta A_{420} = (A_{420}^{d12} - A_{420}^{d0} / A_{420}^{d12}) \times 100$ (1), where A_{420}^{d0} and A_{420}^{d12} were the browning values at the beginning of the treatment and after 12 days, respectively. Similarly, the percentage changes in antioxidant activity ($\% \Delta A_R$), (-)-epicatechin concentration ($\% \Delta E$) and total phenolic content ($\% \Delta TP$) were calculated.

Correlations between the experimental parameters and percentage changes were established with regression analysis at 99.99% significance level.

RESULTS

The accelerated browning test is a reliable test for assessing browning capacity of white wines (Singleton and Kramling, 1976). The conditions employed permitted the examination of samples within a reasonable period of time, although the end point was chosen arbitrarily based on a previous study (Sioumis, Kallithraka, Makris & Kefalas, 2006). A zero order reaction model produced a good fit of the data: $A_{420} = A_{420}^0 + kt$ where A_{420} may be considered the concentration of brown products, A_{420}^0

the initial concentration of brown products, k the reaction rate constant ($A_{420} \times \text{days}^{-1}$) and t time. The k values were calculated from the slope of the regression lines obtained from the graphical representation of A_{420} values versus time and shown in Table 2. As it can be seen from Table 2, the values obtained for k range from 7.4 to 43.3 ($A_{420} \times \text{days}^{-1}$). The wines that showed the lower values regarding k were those made from aidani and asyrtiko. Practically, those wines would develop brown colour later than the other wines examined in this study. On the other hand, the wines with the higher values were those made by monemvasia and malagouzia. Similarly, k values were calculated from the slope of the regression lines obtained from the graphical representations of A_R values versus time and are also shown in Table 2. Antioxidant activity k values ranged from 0.04 to 7.4 ($A_R \times \text{days}^{-1}$).

The total phenolic content of the wines ranged from 197.99 (aidani) to 362.38 (monemvasia) GAE (Table 1). These values are within the range reported for white wines from other studies (Sioumis, Kallithraka, Makris & Kefalas, 2006; Kallithraka, Salaha & Tzourou, 2009). With respect to the antioxidant values, antioxidant activity ranged from 0.87 (malagouzia) to 1.24 (monemvasia) TRE (Table 1).

Since a previous study (Kallithraka, Salaha & Tzourou, 2009), has shown that the concentrations of tartaric acid esters were not significantly affected by the accelerated browning test, this study was focused on flavanol concentration. The following phenolic compounds were identified initially and after 12 days at 55 °C in the white wines studied: (+)-catechin, (-)-epicatechin, and procyanidins B1, B2 and C1. (+)-catechin and procyanidin concentrations were not affected significantly by the browning test while (-)-epicatechin concentration (E) was decreased (Table 2).

Based on the values of A_{420} , A_R , E and TP that were obtained at day 12, the percentage changes were calculated using equation (1) and attempts were made to

distinguish whether or not these parameters could indeed affect the development of brown shades. The assessment was based on regression analysis at 99.99% significance level, and the results drawn are presented analytically in Table 3.

DISCUSSION

Numerous studies have been made on factors affecting the susceptibility of white wines to develop a brown colour in the presence of oxygen and other oxidising agents (e.g. transition-metal ions), yet the examination required for sufficient knowledge of this phenomenon are still incomplete.

In this study it was attempted to examine browning, achieved through an accelerated browning test, in relation to changes in wine oligomeric phenolic composition. Furthermore, in order to better illustrate whether and to what extent oxidation impacts the antioxidant status in white wines, antioxidant activity was determined along with browning development at 24-h intervals for twelve days. It was observed that A_R was increased linearly with time (Table 2). This is in agreement with the findings of a previous study (Kallithraka, Salaha & Tzourou, 2009), where it was observed that accelerated browning increases wine antioxidant activity. However, in the previous study, A_R was determined before and after the end of the accelerated heating treatment and not during the twelve days of the test. Under the present experimental conditions, it became possible to determine the kinetic behaviour of A_R (Table 2).

The existing information on the change of wine antioxidant activity with time is rather conflicting. Whereas some studies (Roginsky, De Beer, Habertson, Kilmartin, Barsukova & Adams, 2006; Zafrilla et al., 2003) indicated that A_R of red wines does not correlate with wine age, some researchers (De Beer, Joubert, Gelderblom & Manley, 2005; Landrault, Poucheret, Ravel, Casc, Cros & Teissedre, 2001; Pellegrini,

Simonetti, Brena, Brifgenti & Pietta, 2000) concluded that the A_R decreases with time. Larrauri, Sánchez-Moreno, Rupérez & Saura-Calixto, (1999), stated that the aged wines had more ability for scavenging free radicals than the young wines. These disagreements indicate the different behavior of wines from different sources produced by different winemaking technologies. In addition, the different methods used for determining A_R in various studies may give inconsistent results. It should be emphasized at this point, that the increased temperature employed for this study, only reduces the amount of time during which measurable browning changes may occur by increasing the rate of the reaction(s) involved (Fernández-Zurbano, Ferreira, Pena, Escudero, Serrano & Cacho, 1995).

As wine develops browning, the oxidative transformation of phenolics results in their condensation and polymerization. However, the number of active $-OH$ groups responsible for A_{AR} remains unchanged (Roginsky, De Beer, Habertson, Kilmartin, Barsukova & Adams, 2006). This is generally true for the condensation of wine phenolics under anaerobic conditions, for instance the condensation induced by acetaldehyde (Waterhouse, 2002). Thus, this could be a possible explanation for the observed increase in A_R of the oxidized wines. In addition, the increase in A_R may be attributed to the higher A_R of the condensation products, such as tannin polymers, since they display a higher A_R than the original monomeric phenols (Roginski, De Beer, Habertson, Kilmartin, Barsukova, & Adams, 2003; Hagerman et. al, 1998). In fact, procyanidins were reported to be 15-30 times more effective at quenching peroxy radicals than simple phenolics or trolox (Hagerman et al., 1998).

The correlations of the selected parameters with kA_{420} showed some significant relationships with a probability at 99.99% significance level (Table 3). In more detail, kA_{420} was positively and significantly correlated with the percentage reduction of (-)-

epicatechin concentration (Figure 1). In previous studies (Kallithraka, Salaha & Tzourou, 2009; Sioumis, Kallithraka, Makris & Kefalas, 2006), it was shown that (-)-epicatechin might be responsible for the development of browning in white wines after accelerated browning test. It was observed that, (-)-epicatechin content of white wines correlated with browning rate and this was explained theoretically by its higher oxidisability (lower oxidation potential) compared with its isomer (+)-catechin (Yang, Kotani, Arai & Kusu, 2001). The results of the present study indicated that browning rate might depend primarily on the amount of (-)-epicatechin which is oxidized rather than on its initial concentration in wine. It appears therefore, that the reactions leading to brown compounds involve *o*-diphenol oxidation, and in particular (-)-epicatechin oxidation plays a crucial role. (-)-epicatechin decrease was also significantly correlated with the increase in A_{420} of the wines (Figure 1).

A significant negative correlation was also obtained between kA_{420} and kA_R (Table 3). The increase in the antiradical activity and its significant correlation with browning increase provided sufficient evidence that the antioxidant status of wines undergoes pronounced changes, which result in an elevation of their antioxidant capacity. One should expect that the oxidation of polyphenols would lead to wines with lower antioxidant capacity, but because reactions between oxidised phenolics may bring about formation of novel antioxidants, an increase in the wine antioxidant status may be observed. However, the rate of browning development is inversely related with the rate of A_{AR} increase (Figure 2). Since browning rate was mainly attributed to (-)-epicatechin loss due its oxidation, it is possible that high (-)-epicatechin losses, are related with reduced rates of A_{AR} increase.

An unexpected observation was the significant negative correlation obtained between $\% \Delta TP$ and kA_{420} . Higher browning rates were observed in samples with lower

differences in total phenolic concentration before and after the heat treatment. This observation might be due to the increasing concentration of *ortho*-quinones which are formed from the oxidation of the *ortho*-dihydroxyphenolic compounds such as (+)-catechin, (-)-epicatechin, caffeic and other hydroxycinnamic acids. These compounds are characterized as pro-oxidants and hence they might be responsible for decreasing the response measured by the Folin-Ciocalteu assay and thus reducing the measured total phenolic concentration. However, as oxidation proceeds, oxidative transformations of phenolics result in their polymerization due to condensation reactions (Guyot, Vercauteren, & Cheynier, 1996). These brown-yellow polymers are possibly responsible for the simultaneous increase in antioxidant activity.

In conclusion, the accelerated browning test resulted in more brown wines with higher antioxidant activity which contained lower concentration of (-)-epicatechin. (-)-epicatechin showed a strong correlation with browning rate. However, the percent reduction of (-)-epicatechin is possibly related with higher browning rates rather than its initial concentration. Finally, the monitoring of the antioxidant activity at 24h intervals, indicated that although increases in browning are accompanied by an increase in the antioxidant activity, the rate of browning development is inversely related with the rate of A_{AR} increase.

REFERENCES

- Arnous, A., Makris, D.P. & Kefalas, P (2001). Effect of principal polyphenolic components in relation to antioxidant characteristics of aged red wines, *Journal of Agricultural and Food Chemistry*, **49**, 5736-5742.
- Benítez, P., Castro, R., Sánchez Pazo, J.A. & Barroso, C.G. (2002). Influence of metallic content of fino sherry wine on its susceptibility to browning, *Journal of Agricultural and Food Chemistry*, **35**, 785-791.
- De Beer, D., Joubert, E., Gelderblom, W.C.A. & Manley (2005). Changes in phenolic composition and antioxidant activity of Pinotage, Cabernet Sauvignon, Chardonnay and Chenin blanc wines during bottle ageing., *South African Journal of Enology and Viticulture*, **26**, 6-15.
- Cilliers J.L. & Singleton V.L. (1990). Nonenzymic autoxidative reactions of caffeic acid in wine, *American Journal of Enology and Viticulture*, **41**, 84-86.
- Fernandez-Zurbano P., Ferreira V. , Pena C. , Escudero A., Serrano F. & Cacho J. (1995). Prediction of oxidative browning in white wines as a function of their chemical composition, *Journal of Agricultural and Food Chemistry*, **43**, 2813-2817.
- Gonzales Cartagena, L., Pérez-Zóñiga, F. J., & Abad, F. B. (1994). Interactions of some environmental and chemical parameters affecting the color attributes of wine. *American Journal of Enology and Viticulture*, **45**, 43–48.
- Guyot, S., Vercauteren, J. & Cheynier, V. (1996). Structural determination of colourless and yellow dimers resulting from (+)-catechin coupling catalysed by grape polyphenoloxidase. *Phytochemistry*, **42**, 1279–1288.

Hagerman, A.E., Riedl, K.M., Jones, A., Sovic, K.N., Ritchard, N.T., Hartzfeld, P.W. & Riechel, T.L. (1998). High molecular weight plant polyphenolics (tannins) as biological antioxidants, *Journal of Agricultural and Food Chemistry*, **46**, 1887-1892.

Kallithraka, S., Salaha, M. & Tzourou, E. (2009) Changes in phenolic composition and antioxidant activity of white wine during bottle storage: Accelerated browning test versus bottle storage, *Journal of Food Chemistry*, **113**, 500-505.

Landrault, N., Poucheret, P., Ravel, P., Gasc, F., Cros, G. & Teissedre, P.L. (2001). Antioxidant capacities and phenolic levels of French wines from different varieties and vintages. *Journal of Agricultural and Food Chemistry*, **49**, 3341-3348.

Larrauri, J.A., Sánchez-Moreno, C., Rupérez, P. & Saura-Calixto, F. (1999) Free radical scavenging capacity in the aging of selected red Spanish wines, *Journal of Agricultural and Food Chemistry*, **47**, 1603-1606.

Makris, D. P., Psarra, E., Kallithraka, S. & Kefalas, P. (2003). The effect of polyphenolic composition as related to antioxidant capacity in white wines. *Food Research International*, **36**, 805–814.

Nagel C.W. & Graber W.D. (1988). Effect of must oxidation on quality of white wines, *American Journal of Enology and Viticulture*, **39**, 1-4.

Pellegrini, N., Simonetti, P., Brenna, O., Brighenti, F. & Pietta, P. (2000). Polyphenol content and total antioxidant activity of Vini novella (young red wines), *Journal of Agricultural and Food Chemistry*, **48**, 732-735.

Psarra, E., Makris, D. P., Kallithraka, S. & Kefalas, P. (2002). Evaluation of the antiradical and reducing properties of selected Greek white wines: correlation with polyphenolic composition, *Journal of the Science of Food and Agriculture*, **82**, 1014–1020.

- Roginski, V., De Beer, D., Habertson, J., Kilmartin, P.A., Barsukova, T. & Adams, D. (2006). The antioxidant activity of Californian red wines does not correlate with wine age, *Journal of the Science of Food and Agriculture*, **86**, 834-840.
- Singleton, V.L. (1987) Oxygen with phenols and related reactions in musts, wines and model systems: Observations and practical implications. *American Journal of Enology and Viticulture*, **38**, 69–77.
- Singleton, V. L. & Kramling, T. E. (1976). Browning of white wines and an accelerated test for browning capacity, *American Journal of Enology and Viticulture*, **27**, 157–160.
- Sioumis, N., Kallithraka, S., Makris, D. & Kefalas, P. (2006) Kinetics of browning onset in white wines: influence of principal redox-active polyphenols and impact on the reducing capacity. *Food Chemistry*, **94**, 98-104.
- Waterhouse, A.L. (2002). Wine phenolics, *Ann NY Acad Sci*, *957*, 21-36.
- Yang, B., Kotani, A., Arai, K., & Kusu, F. (2001). Estimation of the antioxidant activities of flavonoids from their oxidation potentials. *Analytical Sciences*, **17**, 599–604.
- Zafrilla, P., Morillas, J., Mulero, J., Cayuela, J.M., Martinez-Cacha, A., Pardo, F. & López Nicolás, J.M. (2003). Changes during storage in conventional and ecological wine: Phenolic content and antioxidant activity, *Journal of Agricultural and Food Chemistry*, **51**, 4694-4700.

Table 1: Origin and compositional factors of the wines studied

Sample no.	Variety	Origin	TP ¹	A _R ²
1	Malagouzia	Paros	251.54+/-6.63	0.87+/-0.050
2	Monemvasia	Paros	362.38+/-6.76	1.24+/-0.001
3	Malagouzia+	Paros	332.70+/-8.09	1.00+/-0.004
	Asyrtiko			
4	Asyrtiko	Santorini	318.80+/-8.10	1.12+/-0.009
5	Athiri	Santorini	254.56+/-8.90	1.09+/-0.009
6	Aidani	Santorini	197.99+/-3.67	0.91+/-0.010
7	Vilana	Crete	305.42+/-5.42	1.01+/-0.006
8	Athiri	Rhodes	289.65+/-4.36	0.99+/-0.008

¹: Represents total polyphenols expressed as milligrams per liter of gallic acid equivalents (GAE) +/-standard deviation

²: Antioxidant activity expressed as Trolox equivalents (mM trolox) TRE +/-standard deviation

Table 2: Browning ($k A_{420}$) and antiradical activity ($k A_R$) rate constants and percentage changes in browning ($\% \Delta A_{420}$), antiradical activity ($\% \Delta A_R$), (-)-epicatechin concentration ($\% \Delta E$) and total phenolic content ($\% \Delta TP$)

Sample No	$k A_{420}$ ($\text{day}^{-1} \times 10^{-3}$)	$k A_R$ ($\text{day}^{-1} \times 10^{-3}$)	$\% \Delta A_{420}$	$\% \Delta A_R$ ¹	$\% \Delta E$ ²	$\% \Delta TP$ ³
1	41	2,6	84.08	4.55	48.63	15.30
2	43.3	0,04	91.59	0.41	38.36	16.46
3	29.9	2,2	90.63	3.09	38.56	15.35
4	13.9	4.8	49.93	4.45	32.89	22.89
5	15.7	0.3	51.91	2.71	27.57	26.33
6	7.4	7.4	29.63	9.76	20.47	24.16
7	25.2	2.9	70.56	3.85	32.56	18.98
8	13.2	5.1	44.98	2.78	27.69	24.21

¹: Antioxidant activity expressed as Trolox equivalents (mM trolox) TRE

²: (-)-Epicatechin concentration expressed as mg/L

³: Total polyphenols expressed as milligrams per liter of gallic acid equivalents (GAE)

Table 3: Pearson's correlation coefficients¹ calculated after regression analysis between $k_{A_{420}}$ and $\% \Delta A_R$, $\% \Delta E$, $\% \Delta P$ and k_{A_R}

Parameters	$k_{A_{420}}$
$\% \Delta A_R$	n.s. ²
$\% \Delta E$	0.878
$\% \Delta TP$	-0.873
k_{A_R}	-0.856

¹: Correlations are significant at the 0.01 level (two-tailed)

²: Not significant correlations at the 0.01 level