ADDITION OF CARBON DIOXIDE AND ASCORBIC ACID IN WHITE WINES: EFFECTS ON BROWNING DEVELOPMENT AND ANTIOXIDANT ACTIVITY

M., Kyraleou[1], E., Boviatsi[1], N., Proxenia[1], Y., Kotseridis [1], S. Kallithraka [1]

mkyraleoy@yahoo.gr

Abstract

White wines contain low concentrations of antioxidant compounds, which make them sensitive to the oxidation and the development of brown colour. Browning is a serious problem which affects the quality and the sensory attributes of white wines. It is well known that it is associated with polyphenol oxidation, and therefore it may be accompanied by changes in the antioxidant capacity. The addition of different amounts of carbon dioxide (C1:0.535g/L, C2:0.9g/L, C3:1.1g/L) and ascorbic acid (As1:100mg/L, As2:150mg/L, As3:200mg/L) before bottling and the effect in browning capacity and antioxidant protection of white wines was investigated in this work. Browning was approached from a kinetic point of view by the accelerated browning test and antioxidant activity was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH•) assay. The results showed that the percentage change in browning ($\%\Delta A_{420}$) and the antioxidant activity (A_R) were significantly affected by the addition of carbon dioxide and ascorbic acid. Antioxidant activity enhancement was observed after the addition of CO2 and ascorbic acid in all samples.

Keywords: browning rate, antioxidant activity, wine, polyphenols

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 SO_2 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 catalyze 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

[1] Department of Food Science & Technology, Agricultural University of Athens, 75 Iera Odos, 11855 Athens, Greece

both free radical terminators and metal chelators (Benítez, Castro, Sànchez Pazo & Barroso, 2002).

The present study was undertaken to examine the effect of carbon dioxide and ascorbic acid addition on browning development in a white wine produced by a native variety grown in Greece and to evaluate browning rate in relation to its result on antiradical parameters.

MATERIAL AND METHOD

Different amounts of carbon dioxide C1:0.535g/L < C2:0.9g/L < C3:1.1g/L and of ascorbic acid As1:100mg/L< As2:150mg/L<As3:200mg/L were added in wines made from the native Greek variety *V. vinifera* Moshofilero. A control sample (NA) with no addition of CO_2 or As was kept for comparison reasons. The total SO_2 level was set to 100 mg/L prior to bottling.

2.1. 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 ºC 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.

2.2. 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).

2.3. 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.

RESULTS AND DISCUSSIONS

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 the presence of different concentrations of ascorbic acid and carbon dioxide.

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.

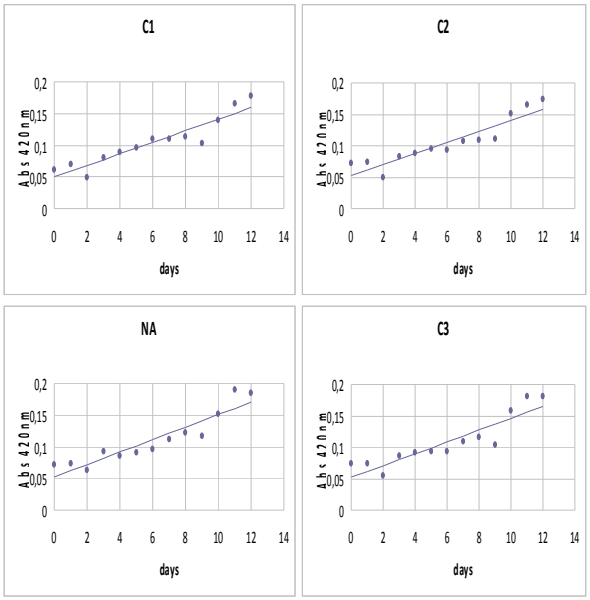
Based on the values of A420 that where obtained at first day and 12th day, the percentage change in browning was calculated as follows %ΔA420 = 100x (A420d12 - A420d0) / A420d12. The %ΔA420 was higher in control sample compared to As1, As2 &As3. However, the sample with the lower concentration of CO2 showed the highest colour change compared to the rest (table 1).

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).

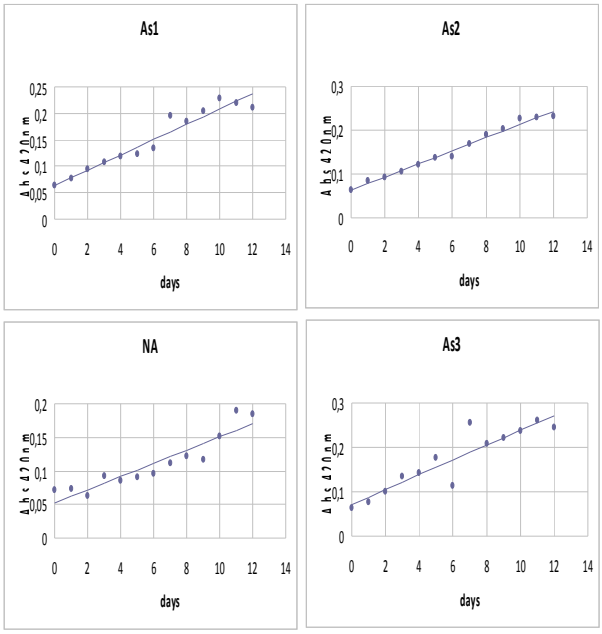
A zero order reaction model produced a good fit of the data: $A_{420} = A_{420}^{0} + kt$ where A_{420} may be considered the concentration of brown products, A_{420}^{0} the initial concentration of brown products, k the reaction rate constant (A_{420} x $days^{-1}$) and t time.

The k values were calculated from the slope of the regression lines obtained from the graphical representation of A_{420} values versus time (Figure 1&2) and shown in Table 1.

1.



2.



Figures 1&2: graphical presentation of the absorption 420nm (y axis) versus time (x axis) during accelerated browning test. NA, blank sample; C1<C2<C3, increasing amounts of CO_2; As1<As2<As3, increasing amounts of ascorbic acid.

As it can be seen, the values obtained for *k* range from 9.1 to 16.7 (A420 x days-1). The wines C1, C2&C3 showed lower browning rates compared to NA. Practically, those wines would develop brown colour later than the other wines examined in this study. In contrast, the wines As1, As2 & As3 showed the higher values (Table 1).

Table 1

Browning (k A_{420}) rate, the percentage changes in browning (%ΔA_{420}) & antiradical activity the 1st and 12th day

sample	% ΔA_{420}	k A_{420}	*Antiradical activity (mM Trolox)*	
			1st day	*12th day*
C1	65.5	9.1	1.52	1.27
C2	58.8	8.8	1.47	1.31
C3	59.6	9.4	1.35	1.36
As1	51.0	14.5	1.42	1.27
As2	53.8	15.1	1.33	1.43
As3	56.1	16.7	1.44	1.25
NA	61.7	9.8	1.38	1.25

Antioxidant activity values ranged from 1.25 to 1.52mM Trolox (Table 1). The addition of CO_2 and ascorbic acid resulted in higher values of antioxidant activity compared to control.

It was observed that A_R was decreased with time (Table 1). This is in disagreement with the findings of a previous study (Kallithraka, Salaha & Tzourou, 2009), where it was observed that accelerated browning increases wine antioxidant activity. 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) 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) concluded that the A_R decreases with time.

CONCLUSIONS

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.

The results showed that the percentage change in browning (%ΔA420) and the antioxidant activity (AR) were significantly affected by the addition of carbon dioxide and ascorbic acid. The addition of ascorbic acid resulted in higher browning rates k but lower %ΔA420. The addition of CO_2 resulted in wines with lower k, but no significant differences observed in the %ΔA420. Antioxidant activity enhancement was observed after the addition of CO_2 and ascorbic acid in all samples.

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 (Fernàndez-Zurbano, Ferreira, Pena, Escudero, Serrano & Cacho, 1995).

REFERENCES

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.

Cilliers J.L. & Singleton V.L., 1990 – *Nonenzymic autoxidative reactions of caffeic acid in wine*, American Journal of Enology and Viticulture, **41**, 84-86.

De Beer, D., Joubert, E., Gelderblom, W.C.A. & Manley, 2005- *Changes in phenolic composition and antioxidant activity of Pinotage, Cabernet Sauvignon, Chardonay and Chenin blanc wines during bottle ageing.,* South African Journal of Enology and Viticulture, **26***,* 6-15.

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-Zúñiga, F. J., & Abad, F. B., 1994 - I*nteractions 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.

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.

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.

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.