Loss of *trans*-resveratrol during storage and ageing of red wines

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Abstract

Background and Aims: The concentration of free *trans*-resveratrol in wine is expected to increase with storage because of hydrolysis of piceid (a bound form of resveratrol). This study measured resveratrol concentration in 16 Australasian red wines ranging from 1 to 6 years old, both initially and after a storage period of 16 months under ambient conditions.

Methods and Results: During the study period, *trans*-resveratrol concentration decreased by an average of 76%. The decay in *trans*-resveratrol was first order over the tested range of wine source and vintage, with a mean *trans*-resveratrol bottle-storage half-life of 8 (±1 SD) months. Decay rate was remarkably independent of growing condition and year.

Conclusion: The data suggest isomerisation of *trans*- to *cis*-resveratrol via residual enzymatic activity (rather than being catalysed by light or acid).

Significance of the Study: In general, this may reduce the anticipated health benefits of the wine given that *cis*-resveratrol displays fewer health benefits than the *trans* isomer.

Keywords: *health benefits, HPLC, piceid [-3-(β-D-glucopyranoside)], residual enzymatic activity, resveratrol*

Introduction

Resveratrol [3,5,4′-trihydroxystilbene (Figure 1)] is a phytoalexin produced by plants as a means of defence against pathogens and herbivores (Lattanzio et al. 2006, van Baarlen et al. 2007, Adrian and Jeandet 2012). Structurally, it is a stilbene derivative occurring in both *cis* and *trans* forms. Because of the presence of its multiple phenolic groups, it possesses greater antioxidant activity than other common antioxidants such as α-tocopherol (Fabris et al. 2008, Gülçin 2010) and hence may provide beneficial health effects to humans, including cardioprotective, anti-inflammatory and anti-diabetic effects (Marques et al. 2009, Mukherjee et al. 2010, Fabjanowicz et al. 2018). In comparison to other foods, a typically higher concentration of resveratrol is found in grape and berry skins and hence is extracted in the red winemaking process (Siemann and Creasy 1992). In synergy with other phenolic substances, resveratrol is one of the major reasons for the perceived health benefits of red wine (Xiang et al. 2014).

As the effects of resveratrol are dose-dependent (Mukherjee et al. 2010), determining changes in resveratrol concentration with the storage of wines is of prime interest in determining any potential alteration in the perceived health benefits of red wines (Xiang et al. 2014). In red wines, resveratrol is primarily present bound to various sugars, such as resveratrol-3-(β-D-glucopyranoside) (Mattivi et al. 1995, Ribeiro de Lima et al. 1999). With the natural acidic hydrolysis at storage temperature of these sugar–resveratrol complexes over time, the free resveratrol concentration in wines would be expected to increase with storage. In this study, we investigated changes in the concentration of *trans*-resveratrol across 16 red wines.

Materials and methods

Sixteen wines originating from Australia and New Zealand were sourced from commercial suppliers, with production years ranging from 2007 to 2012. Further details are provided in Table 1. *Trans*-resveratrol concentration was determined by reversed-phase HPLC (Siemann and Creasy 1992, Yong and Hui 2011). A Shimadzu HPLC-PDA system (Rydalmere, NSW, Australia) was used with a C_{18} column [Luna; 150 × 4.6 mm i.d.; 3 µm particle size (Phenomenex, Torrance, CA, USA)]. The mobile phase consisted of 70% Milli-Q water (Merck Millipore, Macquarie Park, NSW, Australia), 29.9% acetonitrile and 0.1% glacial acetic acid at a flow rate of 1 mL/min and a column temperature of 30°C. The injection volume was 20 µL, with the resveratrol peak quantified at 306 nm. Samples were syringe filtered (0.45 µm cellulose filters) prior to analysis. To create the resveratrol standards, a control wine was spiked with resveratrol stock (made up in 12% ethanol) at a concentration ranging between 0.1 and 6.0 mg/L. All reagents were obtained from Sigma-Aldrich Australia (Castle Hill, NSW, Australia). The presence of *trans*-resveratrol was confirmed in each extract by comparison of its retention time (7.6 min) and UV spectral characteristics, with a reference standard run in the same sequence. Prior to analysis, samples were sonicated and syringe filtered [Millipore 0.45 µm cellulose ester (Merck Millipore)]. Exposure to air and light was minimised to prevent degradation of the wine constituents. Each sample was run in duplicate.

After measurement of the initial resveratrol concentration of the wines in July 2013, the wines were N_2 flushed, resealed and stored at ambient temperature in darkness. To provide the most realistic storage conditions, the original packaging of the

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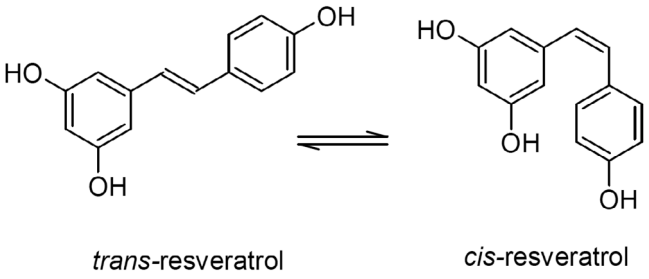

Figure 1. The structural formula of *trans*-resveratrol and *cis*-resveratrol.

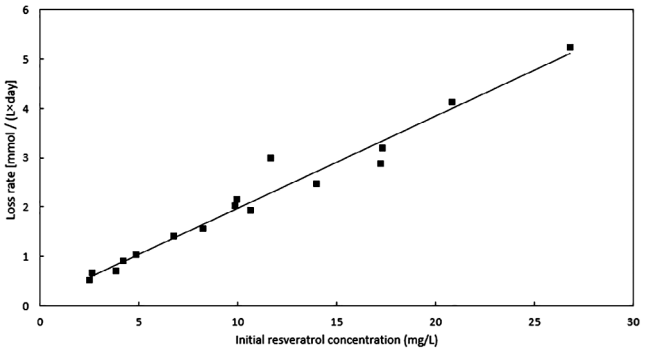

Figure 2. Relationship between the initial resveratrol concentration in each wine and its corresponding loss rate. A linear regression fitted to the data ($y = 0.186x + 0.122$) gave a good fit ($R^2 = 0.966$).

wine was used. The resveratrol concentration was again measured in November 2015, after 16 months of storage.

Statistical analysis was performed in IBM SPSS (SPSS, Chicago, IL, USA). All data collected were normally distributed according to both Kolmogorov–Smirnov and Shapiro–Wilk testing ($P > 0.05$); therefore, parametric testing was used throughout.

Results and discussion

There was no clear relationship between production year and initial resveratrol concentration of the wines. The average resveratrol concentration of the wines decreased from 10.7 ± 7.0 mg/L (mean $\pm$ 1 SD) in July 2013 to 2.8 ± 2.3 mg/L (mean $\pm$ 1 SD) in November 2015 (n = 16 for both). The reduction in resveratrol concentration ranged from 63 to 96%, with an average reduction of $76 \pm 9\%$ (n = 16). This reduction was statistically significant according to a paired-samples t test ($t_{15} = 6.365$, $P < 0.001$). This observation contrasted with that of Jeandet et al. (1995), who suggested that resveratrol concentration was relatively stable in three varieties of aged French wine. The average amount of resveratrol found in their study (2.1 mg/L), however, was much lower than the initial amount found in the wines of the present study, suggesting the possibility that their wines may have already undergone an initial loss of resveratrol before reaching a stable concentration.

There was a linear relationship between the initial resveratrol concentration in the wine and its loss rate ($R^2 = 0.966$), with samples having a higher initial resveratrol concentration displaying a more rapid loss of resveratrol.

The data presented here confirm that the resveratrol concentration of aged red wine decreases with storage time. This is most likely because of isomerisation of *trans*-resveratrol to the *cis* configuration, a major process during vinification (Mattivi et al. 1995) and in oak-barrel storage (Hernández et al. 2006), which is likely to continue during bottle storage. Owing to the lack of a *cis*-resveratrol standard at the time of analysis, this could not be confirmed via HPLC. López-Hernández et al. (2007), however, found a mean ratio of *trans:cis* resveratrol of 2.7:1 in Spanish white wine approximately 2 years of age. This correlates with the ~75% reduction in *trans*-resveratrol concentration observed in this study, indicating that the equilibrium reaction between *trans* and *cis* isomers had proceeded to near completion. Also of support is the notably low *trans*-resveratrol concentration after storage (Table 1).

The data (Figure 2) are well correlated to first-order decay of *trans*-resveratrol with a mean half-life of $t_{1/2} \approx 8$ ($\pm$1, SD) months. That the half-life of the samples varied so little with their source and vintage suggests a common mechanism. As the samples were stored in brown glass bottles in the dark, this argues against the previously reported UV/light-induced *cis-trans* isomerisation mechanism (Deak and Falk 2003); rather, it is, as previously suggested for oak-barrel ageing (Hernández et al. 2006), a result of a residual enzymatic activity. Acid-catalysed isomerisation, if any

Table 1. *Trans*-resveratrol concentration in the studied wines in July 2013 and November 2015.

	Resveratrol concentration (mg/L)		
Wine	**2013**	**2015**	**Reduction (%)**
Grampians Merlot (2008)	2.48	0.53	78.6
Barossa Cabernet Sauvignon (2010)	2.65	0.15	94.3
Barossa Shiraz (2010)	3.85	1.18	69.4
Western Australia Cabernet Sauvignon (2010)	4.22	0.82	80.6
Hunter Valley Cabernet (2012)	4.87	0.98	79.9
Ballarat Pinot Noir (2010)	6.76	1.50	77.8
Ballarat Merlot (2010)	8.23	2.39	71.0
Ballarat Cabernet (2010)	9.93	1.89	81.0
Barossa Merlot (2010)	9.86	2.27	77.0
New Zealand Syrah (2008)	10.64	3.40	68.0
Western Australia Shiraz (2011)	11.64	0.42	96.4
Tasmania Pinot Noir (2012)	13.96	4.74	66.0
New Zealand Merlot (2007)	17.21	6.42	62.7
Ballarat Shiraz (2010)	17.31	5.35	69.1
Grampians Pinot Noir (2010)	20.84	5.39	74.1
Grampians Shiraz (2010)	26.80	7.23	73.0
Mean $\pm$ 1 SD (n = 16)	10.70 ± 7.01	2.79 ± 2.32	76.2 ± 9.3

occurs during storage, must play a minor role as it would augment the thermodynamic (i.e. *trans*) isomer (Deak and Falk 2003).

In general, *cis*-resveratrol shows fewer health effects than *trans*-resveratrol (Orallo 2005, 2006), including its anti-inflammatory power (Rius et al. 2010). Hence, it is possible that older red wines will not provide the same degree of health benefits compared to younger red wines. With more recent research, however, questioning the extent to which resveratrol contributes to the health benefits of wine (Xiang et al. 2014), this may not necessarily be the case. It should also be noted that freshly bottled wines may not show the same loss rate of resveratrol as that observed in aged wines as their residual enzymatic activity may change over time. For example, Jeandet et al. (1995) reported that resveratrol was relatively stable in three varieties of aged French wine, in contrast to the marked decrease found here. Hence, further work is required to confirm the dependency of resveratrol flux on the age of the wine in question.

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