

Shedding light on the modulation of key Riesling wine aroma compounds in a changing climate

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Abstract

As Riesling ages, there exists a delicate balance between the loss of young fresh and fruity characters, and the formation of aged notes, including ‘kerosene’ due to 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN). Early formation of ‘aged’ notes in young wine can lead to unbalanced wines that are not necessarily appreciated by consumers. The vineyard drivers associated with earlier evolution of TDN have been examined in this work, along with glycosylated precursors, to aid in better understanding of TDN formation in grapes and wine.

Two commercial vineyards with a difference in temperature of around 2 °C underwent treatments to modulate light exposure to the grape bunches, yielding significant difference in total TDN and providing information into the roles of light and temperature in TDN formation. These treatments allowed for LC-MS/MS studies into glycosidic precursors and tentative identification of several compounds that are expected to contribute to higher amounts of TDN.

Introduction

Characters of young Riesling wine like estery, citrus and floral result from compounds such as monoterpenes or 2-phenylethyl acetate. As Riesling ages these diminish and the wine develops lime, caramel, and kerosene notes. One of the compounds responsible for aged Riesling character, and the ‘kerosene’ descriptor, is 1,1,6-trimethyl-1,2-dihydronaphthalene, or TDN [1]. With a sensory threshold of 2 µg/L [2], TDN can be polarising with sensory intensity not always relating to concentration [3]. While considered important to aged Riesling, it can result in an unbalanced wine especially if it starts to emerge in younger wines and dominate delicate floral or fruity notes.

The evolution of TDN in wine is thought to occur via the breakdown of carotenoids and through reaction and rearrangement of norisoprenoids, which are present in grapes as glycosidically bound species. As such, TDN itself is not present in grapes but forms and accumulates as wine ages. Considered a thermodynamic end-point, TDN is very stable, unlike the monoterpenes responsible for young Riesling characters which degrade as wine ages [4]. Due to light-induced changes in carotenoid profiles during grape growing and structural diversity of the carotenoid end group from which the C13-norisoprenoids are formed, elucidation of the exact carotenoid(s) that give rise to TDN, and hence the pathway to formation, has proved difficult so far.

It is well understood that the timing and intensity of light exposure of grapes modulates the amount of TDN produced in wine [5], but recently it has been proposed that as growing seasons get warmer due to climate change, increasing temperatures result in kerosene notes being more prevalent in younger Riesling wines [6]. Currently, there exists evidence that winemaking practices can be useful in managing the amount of TDN present in a wine, including yeast choice [7], wine pH [8], and closure type (Figure 1) [3].

But the question remains to be answered: is limiting TDN evolution using winemaking interventions is just a case of too little, too late?

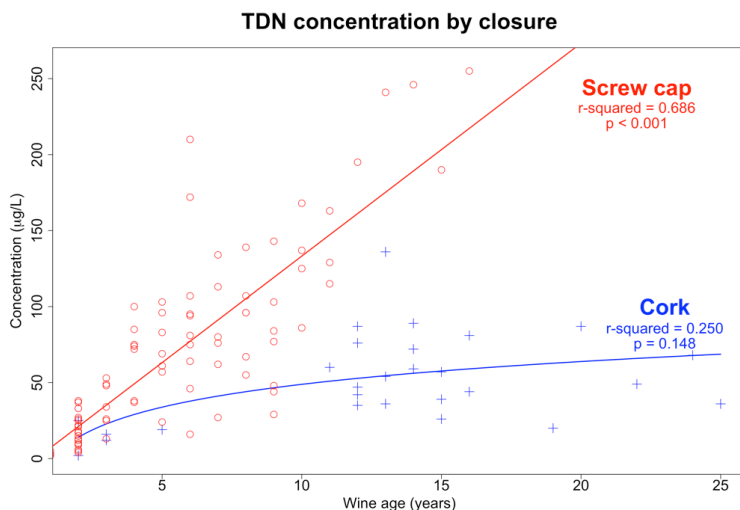


Figure 1: The evolution of TDN in 116 commercial Riesling wines, separated into those with screw cap closures (red circles) and cork closures (blue crosses).

The biggest questions that remain surround the key driving forces in the vineyard that determine the speed and extent of TDN production as the wine ages. Is the changing climate a driving force that will result in Riesling obtaining aged characters earlier in future, and do we continue to employ viticultural techniques to modify light exposure of grapes as a means for achieving optimal maturity? This work is the first step in determining practical solutions for managing TDN concentrations in wine, including a better understanding of the pathway(s) by which TDN is formed, identifying markers in grapes that allow us to predict TDN formation in wine, and ascertaining the true vineyard-based driving forces of high TDN wines.

Experimental

Trial sites and grape sampling

During the 2014/15 growing season, trials were conducted in two commercial vineyards in Barossa Valley (BV) and Eden Valley (EV) of South Australia, separated by 13.7 km, 180 m of altitude, and a mean January temperature differential of 1.94 °C. Treatments were applied 30-days past berry set (11 December for EV, 23 December for BV), where one-third of the bunch zone leaves were removed (leaf-plucked), compared with a control, both replicated 6 times in an alternating manner within two adjacent rows. Within these treatments, light exclusion boxes were applied to single bunches (plucked boxed and control boxed), and temperature was monitored in both canopy and boxes (Tinytag Transit 2 temperature loggers). At commercial harvest (11 February for BV, 19 February for EV) grapes were hand-picked. Grape berries were immediately plucked and randomised. Grapes (300 g) were homogenised (20 s, 8,000 rpm, Retsch Grindomix GM200) and the homogenate stored at -20 °C until further use. When required, the homogenate was thawed, centrifuged and the supernatant used for analyses.

Analysis of total TDN in grape samples

The analysis of total TDN was based on the solid-phase extraction (SPE) protocol described by Kwasniewski et al. [5], using d_8 -naphthalene (Sigma-Aldrich) as the internal standard. Quantification of TDN in the hydrolysed samples using GC-MS was based on the report of Daniel et al. [8], with minor modifications to the oven parameters.

Preparative HPLC of glycosidic material

Based on a reported analytical-scale SPE isolation [9], glycosidic material was isolated from 50 mL of Eden Valley grape homogenate supernatant using 25 SPE cartridges (2 mL per cartridge), to increase scale. The pooled material was concentrated to approximately 2 mL and made up to 4 mL using milli-Q water. An aliquot was diluted 1:10 and 500 μ L injected on a Dionex UltiMate® 3000 Binary Semi-preparative HPLC-DAD system and separation achieved on a Synergi Hydro-RP column (80 Å, 4 μ m, 250 x 21.2 mm) with a C18 guard column (15 x 21.2 mm). A binary gradient with mobile phases consisting of 0.1 % acetic acid in water (A) and 0.1 % acetic acid in acetonitrile (B) with a flow rate of 8 mL/min and elution profile starting at 5% B, increasing linearly to 15% B over 10 mins, then increasing to 30% B over 40 mins, then to 90% B over one minute and held for further 19 mins. The gradient was reduced to 5% B over 1 minute and re-equilibrated for 40 minutes. The column effluent passed through a diode array detector (190-400 nm) and then into a fraction collector. Fractions were collected every 30 seconds, with those representing one peak combined, as were consecutive fractions representing no DAD peak. These were concentrated to dryness and reconstituted in 1 mL of water. Half of this (0.5 mL) was used for LC-MS/MS investigations and half (0.5 mL) analysed for total TDN (as above).

LC-MS/MS investigation into glycosidic TDN precursors

Potential glycosidic precursors to TDN were investigated using a Liquid Chromatography Quadrupole Time-of-Flight-system (Agilent 1200 series LC-system). Due to concentration factors 10 μ L were injected for the exposed and 5 μ L were injected for the control samples, and separation was carried out on a Kinetex PFP column (100Å, 2.7 μ m, 150 x 2.1 mm) using the same mobile phases as above. A flow rate of 0.2 mL/min was used and an elution profile starting at 5% B, increasing linearly to 15% B over 7 mins, then increasing to 30% B over 13 mins, then to 90% B over 12 minutes and held for further 5 mins. The gradient was dropped back to 5% B over 1 minute and then re-equilibrated for 19 minutes. A Bruker micrOTOF-Q II mass spectrometer equipped with an orthogonal ESI source was used for high resolution mass spectrometric analysis. The ionisation was in negative APCI mode with nitrogen curtain, nebulizer and collision gas. The instrument conditions were: capillary voltage (3500 V), end plate offset (-500 V), drying gas (4 L/min, 250 °C), nebulizer gas pressure (0.4 bar); mass scan range (50–1650), and ramped collision energy. External instrument calibration was using sodium formate solution (10 mM NaOH in isopropanol/0.2% formic acid (1:1)).

Statistics and graphics

All graphing and statistical analyses were carried out with the open source statistical programming language R, using custom scripts.

Results and discussion

The two sites represented a climatic shift of approximately 2 °C (1.94 °C based on our temperature data), and provide a good model for the temperature increase predicted under climate change scenarios [10]. The treatments represented extreme defoliation to

increase light exposure and confirm the previous findings correlating exposure and TDN production, plus boxed treatments to create a negative control, no-light scenario. The changes in total TDN brought about by the light modulating treatments were marked (Figure 2), although no significant inter-site variation was observed.

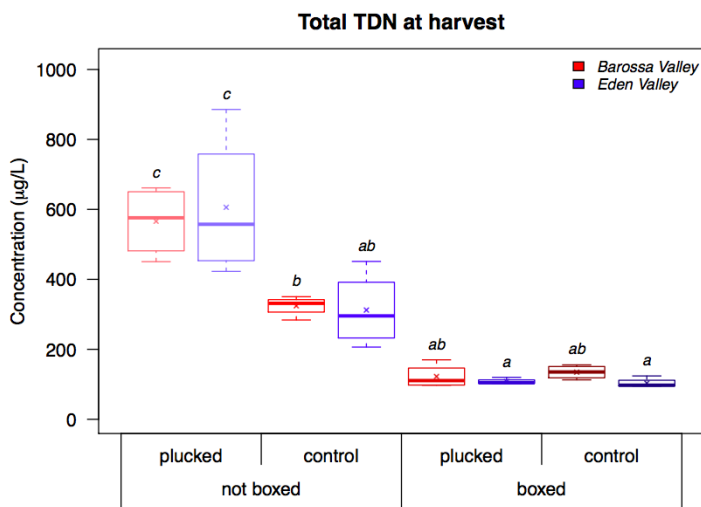


Figure 2: Total TDN in grape homogenates from each of the treatments and sites. Different letter denotes significant difference between treatments ($P = 0.05$).

This confirms the previous findings that light is important in modulating the amount of potential TDN that can evolve as a wine ages [5], but also shows no obvious difference between the two sites that differed by approximately 2 °C. This does not necessarily mean that temperature has no effect, as differences between the vineyards (e.g. trellising, soil, humidity) could be offsetting any temperature effect. Although, this result does imply that increases in grape growing temperature does not necessarily have to result in an increase in TDN production in wine.

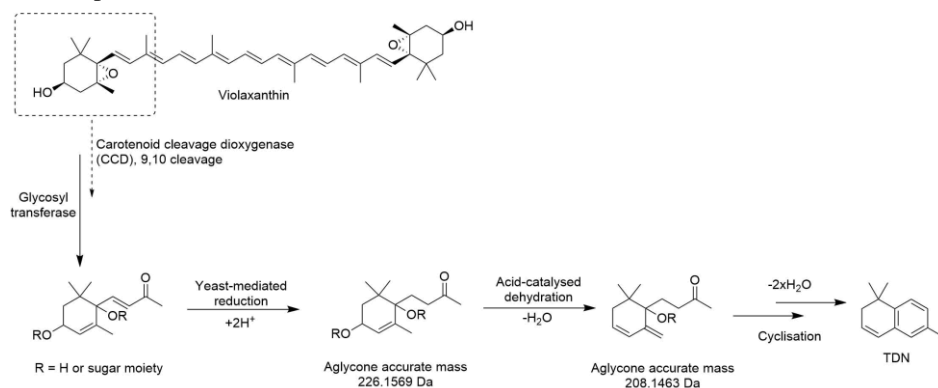


Figure 3: Carotenoid cleavage and one proposed pathway for production of TDN via glycosylated precursors, showing accurate masses for protonated intermediates in this proposed pathway [4, 7]

The C13-norisoprenoids that result from carotenoid cleavage are generally bound to sugars in grapes. Hence, the leaf-plucked treatments provided a means to better

understand TDN production as sun-exposed grapes are expected to contain an increased amount of the glycosylated precursors within the TDN formation pathway. As such, glycosidic fractions were isolated from the Eden Valley control and leaf-plucked grapes, then fractionated using preparative HPLC. The fractions that were collected were then divided, and one half used to determine the total TDN content, to ascertain the potential of the compounds present in that fraction to give rise to TDN, and the other half kept for future LC-MS/MS investigation of fractions of interest.

Although several HPLC-separated fractions gave rise to TDN under hydrolysis conditions, some gave significantly higher proportions from leaf-plucked grapes than from control grapes, and hence were targeted for understanding the up-regulation of TDN formation. Although pooling fractions and differences in dilution makes comparison between fractions and with absolute total TDN values obtained in whole grapes hard, the relative amounts between leaf-plucked and control samples could be determined. Fraction 32 showed the highest concentration of total TDN, and a relative ratio of 3.1 between the leaf-plucked and control samples. Fraction 30 gave a 1.5-fold increase in the leaf-plucked samples, and the pooled fractions 37-50 a 2.6-fold increase. These three fractions were taken through to LC-MS/MS analysis to better understand the compounds present that were giving rise to increased TDN when hydrolysed.

For LC-MS/MS investigation, the masses of hydroxylated compounds (possible aglycones) that are present in the proposed TDN formation pathways (Figure 3) [4, 7] were combined with the known masses of the sugars that predominate in grapes [9, 11, 12] (as well as the potential acetate analogues) to produce a table of masses of interest. A number of ions were identified that fit our requirements: they were present in both treatments; more abundant in the leaf-plucked samples; and equivalent to a mass of interest (Table 1).

Table 1: Ions observed in LC-MS/MS experiments present in higher abundance in leaf-plucked samples and relating to glycosidically bound masses of interest in the proposed TDN formation pathway.

<i>Ion [M+CH₃OO]⁻ (Da)</i>	<i>Fraction</i>	<i>Retention time (min)</i>		<i>Matching structure/mass of interest</i>
		<i>Control</i>	<i>Leaf-plucked</i>	
447.2230	32	12.0	11.8	m/z = 226.1569 + hexose
561.2547	37-50	14.5	14.6	m/z = 208.1463 + hexose + pentose
593.2909	30	12.7	12.6	m/z = 226.1569 + rutinose

Figure 4 shows an example fragmentation pattern from precursor ion 593.2809 Da in the leaf-plucked sample. Here, the acetate adduct ion fragments (-60 Da) to yield the mass of the proposed rutinoid (~553.26 Da), composed of an aglycone mass that is commonly observed in the proposed TDN formation pathways (226.1569 Da), and rutinose. This fragments further via a neutral loss of the aglycone (226.1566 Da). These fragments align with product ion spectra previously observed for guaiacol rutinoid in grapes [11].

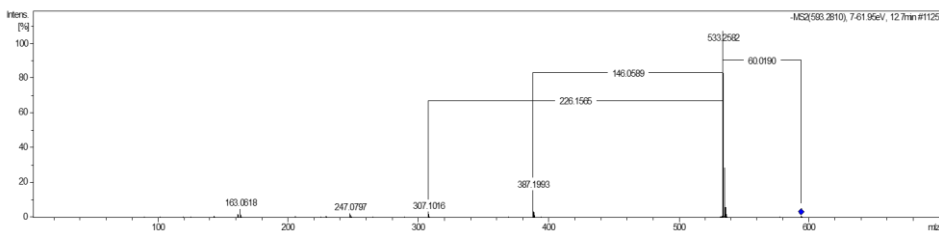


Figure 4: Fragmentation pattern for the ion 593.2809 Da in the leaf-plucked sample.

In summary, these viticultural trials have confirmed the importance of light exposure for increasing the total TDN content of grapes, and shown no significant difference in grapes from two vineyards with an approximate 2 °C growing season difference. Preparative HPLC separation allowed for the LC-MS/MS identification of numerous ions that are more abundant in leaf-plucked samples, with tentative elucidation including disaccharide bound norisoprenoids. These structures will provide a starting point for in depth studies into the formation pathway of TDN in wine.

Acknowledgements

The authors acknowledge collaborators Prof. Drs Doris Rauhut and Manfred Stoll, funding from The Government of South Australia and Wine Australia, and industry partners.

References

1. Simpson, R.F. (1978) *Chemistry and Industry*. p37.
2. Sacks, G.L., Gates, M.J., Ferry, F.X., Lavin, E.H., Kurtz, A.J. and Acree, T.E. (2012) *J. Agric. Food Chem.* 6: 2998-3004.
3. Black, C.A., Francis, I.L., Henschke, P., Capone, D.L., Anderson, S., Day, M., Holt, H., Pearson, W., Herderich, M.J. and Johnson, D. (2012) *Wine and Vitic. J.* 27: .20-26.
4. Black, C.A., Parker, M., Siebert, T.E., Capone, D.L. and Francis, I.L. (2015) *Aust. J. Grape and Wine Res.* 21: 582-600.
5. Kwasniewski, M.T., Vanden Heuvel, J.E., Pan, B.S. and Sacks, G.L. (2010) *J. Agric. Food Chem.* 58: 6841-9.
6. Winterhalter, P. and Gök, R. (2013) in *Carotenoid Cleavage Products*. ACS Symposium Series 1134, Washington, DC, USA. p. 125-137.
7. Gök, R. (2015) PhD. Thesis. Technical University of Braunschweig.
8. Daniel, M.A., Capone, D.L., Sefton, M.A. and Elsey, G.M. (2009) *Aust. J. Grape and Wine Res.* 15: 93-96.
9. Hayasaka, Y., Baldock, G.A., Pardon, K.H., Jeffery, D.W. and Herderich, M.J. (2010) *J. Agric. Food Chem.* 58: 2076-81.
10. IPCC (2014) *Climate Change 2014: Synthesis Report* [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.
11. Hayasaka, Y., Baldock, G.A., Parker, M., Pardon, K.H., Black, C.A., Herderich, M.J. and Jeffery, D.W. (2010) *J. Agric. Food Chem.* 58: 10989-10998.
12. Parker, M., Black, C.A., Barker, A., Pearson, W., Hayasaka, Y. and Francis, I.L. (2017) *Food Chem.* 232: 413-424.