WINE ACTIVE COMPOUNDS 2014

Proceedings of the Third Edition of the International Conference Series on Wine Active Compounds

Edited by Pr. Régis Gougeon
Université de Bourgogne

WAC 2014

March 26, 27, 28, 2014

Sous le patronage de l'Organisation Internationale de la Vigne et du Vin

Oxidative *vs* reductive skin maceration on thiol precursors

Roberto Larcher[a], Loris Tonidandel[a], Giorgio Nicolini[a], Tomas R. Villegas[a], Mario Malacarne[a], Bruno Fedrizzi[b]

[a] *FEM-IASMA Fondazione Edmund Mach Istituto Agrario di San Michele all'Adige, via E. Mach 1, 38010 San Michele all'Adige, TN, Italy*
[b] *Wine Science Programme, School of Chemical Sciences, The University of Auckland, Private Bag 92019, Auckland, New Zealand*

Keywords: skin maceration; thiol precursors; LC-MS;

1. INTRODUCTION

Tropical thiols and their precursors is currently one of the most appealing research topics in wine science [1]. The recent finding that pre-fermentative processes could strongly influence the final concentration of free thiols in wine [2] has put further emphasis on the effect of technological steps, from grape harvest [3, 4] to grape pressing [5], on the content of these molecules.
Particularly interesting is the current dilemma on the effect of oxidative vs reductive conditions. Studies carried out in Australia showed how mechanical harvest (considered to be an oxidative way of handling the grapes) always caused a significant increase of the *S*-glutathionylated and *S*-cysteinylated precursors [4]. A following study [5] showed how the mechanisms involved in the production of pre-fermentative thiol precursors is much more complex and is depended on other unknown variables. In this particular study we are presenting the thiol precursors variability associated with two grape varieties submitted to oxidative and reductive maceration.

2. MATERIALS AND METHODS

2.1 Chemicals and materials

(R/S)-3-S-cysteinylhexan-1-ol (Cys-3MH), (R/S)-glutathionylhexan-1-ol (GSH-3MH) and their labelled forms d_3-(R/S)-3-S-cysteinylhexan-1-ol (d_3-Cys-3MH), and d_3-(R/S)-glutathionylhexan-1-ol (d_3-GSH-3MH) were supplied by Buchem B.V. (Apeldoorn, The Netherlands). Formic acid (FA) and acetonitrile (ACN) of HPLC grade, anhydrous ≥ 99% dimethyl carbonate, ≥ 98% reduced glutathione (GSH), ≥ 97% potassium metabisulfite, ≥ 98% L-ascorbic acid were provided by Sigma-Aldrich (Milan, Italy).
Six kg of sound and technologically ripe grapes of Mueller-Thurgau (MT; N=19) and Sauvignon blanc (SB; 32) were harvested in Trentino (North Italy) in 2012. For each sample the bunches were cut in 3-berries clusters, randomly subdivided in two homogeneous subsamples of 2 kg, and collected in polyethylene plastic bags (40 × 40 cm, for domestic under-vacuum food packaging). Each one of the two subsamples was alternatively submitted to a reductive (RD) or to an oxidative (OX) protocol of skin-contact maceration. In the RD treatment, potassium metabisulfite (320 mg), L-ascorbic acid (160 mg), and dimethyl carbonate (400 mg) were added to the berries, while in the OX protocol no adjuvants were added to the grapes. The RD bags were sealed under partial vacuum in order to remove the majority of the air, carefully avoiding any damage of the berries, while the OX bags were left open. Both the RD and OX grapes were then manually crushed and let in skin-contact maceration at 20°C for 24 hours. Afterwards, 5 mL of must was collected from each sample, if necessary piercing the close bags with a 10-mL plastic medical syringe. The must was then rapidly diluted with 20 mL methanol chilled at – 20°C and added of d_3-GSH-3MH and d_3-Cys-3MH (both at 35 µg L^{-1}) as internal standards. The sample was filtered on a 0.22 µm PVDF syringe cartridge (Millex-GV; Millipore, Tullagreen, Ireland) and stored in 2 mL glass vial at –20°C until analysis.

2.2 LC-MS

The precursors were analysed using an UPLC Acquity (Waters Corporation, Milford, US), connected with the Xevo TQ MS mass spectrometer (Waters). 5 µL sample was

injected on an Acquity UPLC HSS T3 C18 column (2.1 mm × 100 mm, Waters), set at 40°C, and the flow rate was set at 0.45 mL min^{-1}. The eluents were water (A) and ACN (B), both added of 0.1% formic acid. The MS experiments were carried out in positive ion mode. Method linearity for the precursors was studied between 0.1 and 30 µg L^{-1} and the R^2 for the deuterated precursors were always > 0.98.

3. RESULTS AND DISCUSSION

3.1. Juice composition

The juices were collected by manually squeezing the plastic bags; their basic composition for both MT and SB juices showed large variability, nevertheless it was generally in agreement with those found in literature for the same cultivars. This variability is in agreement with the remarkable differences in sites altitude (250–750 m above sea level) and in sunlight exposure in this mountain region. Within-sample variability for total soluble solids was about 4.8°Brix for MT, and about 6.4°Brix for SB. Confirmation of the effectiveness of the experimental protocol in protecting maceration from oxygen, was gained by measuring GSH: the RD treatment produced a GSH concentration between 1.6 and 28.7 mg L^{-1} in the MT juice and 4.4–45.4 mg L^{-1} in the SB juice. As expected, the GSH concentration was significantly ($p < 0.001$) impacted by the OX treatment, with a negligible content in the OX juices for MT and below 10 mg L^{-1} for SB.

3.2. 3-S-cysteinyl and 3-S-glutathionyl 3MH

3MH precursor contents in MT and SB juices treated according to the RD and OX maceration protocols are reported in figure 1.

The concentration range (µg L^{-1}; as min, median and max, respectively) for MT/OX juices was: GSH-3MH = 48, 114, 290; Cys-3MH = 11, 28, 107, and for MT/RD: GSH-3MH = 26, 80, 267; Cys-3MH = 10, 23, 101. These contents are in agreement with those found by Roland et al. [6] in the parent Riesling, and similar to those of Sauvignon Blanc juices. For SB/OX the concentration (µg L^{-1}; min, median and max, respectively) was: GSH-3MH = 143, 209, 577; Cys-3MH = 30, 82, 310, and for SB/RD: GSH-3MH = 118, 201, 568; Cys-3MH = 28, 79, 273. These values are in agreement with those of Capone and Jeffery [4] but remarkably higher than those of Allen et al. [3]. Our results seem generally to confirm what was observed by Roland and colleagues [6] on the capability of controlled addition of oxygen into micro-fermentations to increase the final concentration of GSH-3MH. The OX treatment increased GSH-3MH and Cys-3MH in 16 and 13, respectively, of the 19 MT juice samples, and in 23 and 20, respectively, of the 32 SB juice samples. The effect of the OX treatment on juice composition was verified using parametrical (Tukey's HSDT) and non-parametrical (Wilcoxon matched-pairs test) tests. Significant differences were found for GSH-3MH when using the non-parametrical approach, with mean values higher for the OX treatment, whilst no differences were found for Cys-3MH. Even though most samples had a higher GSH-3MH concentration under the OX protocol (84% in MT and 72% in SB), other showed an opposite behavior. Similar evolution was observed for Cys-3MH, with the percentage equal to 68% in MT and 63% in SB. GSH and either of the thiol precursors did not show any correlations for the trials; a more complicated pathway could be hypothesised. These results are similar to those reported by Roland et al. [6] where a significant increase for GSH-3MH was measured in oxidative environments even though that study considered only one sample per variety.

The content ratio Cys-3MH/GSH-3MH (calculated from the precursor mean concentrations) for MT was 0.25 and 0.30 for OX and RD, respectively; while for SB was 0.43 and 0.44, respectively. For SB the values were in both OX and RD treatments about ten times lower than those found by Roland et al. [7]. The OX and RD treatments significantly (Tukey's HSDT; $p < 0.05$) influenced the precursor concentration ratio only for MT.
No correlations between the basic juice composition and the thiol precursor abundance for both reductive and oxidative treatments were observed for this study.
Concluding, OX maceration promoted higher content of GSH-3MH, particularly in MT, whilst Cys-3MH showed to be independent on the oxygenation of musts in this phase.
This work confirms once more that the *de novo* synthesis of the 3-MH precursors can

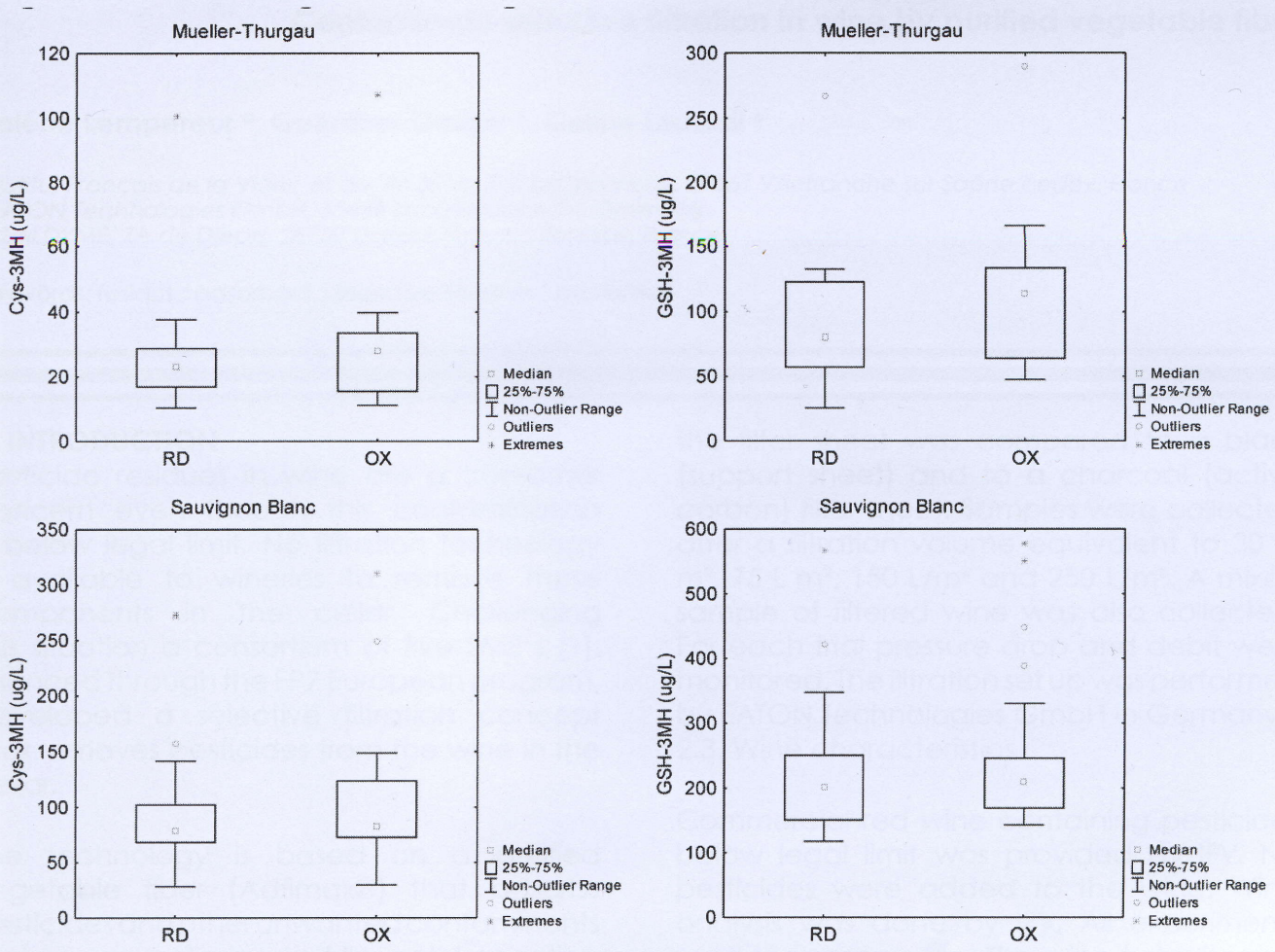

Table 1. GSH-3MH and Cys-3MH content in 58 commercial juices grouped by botanical origin.

really take place during maceration or grape machine harvesting, nonetheless no clear strategy can be suggested for a general optimization of the winemaking processes.

References.

1. A. Roland, R. Schneider, A. Razungles, F. Cavelier, Varietal Thiols in Wine: Discovery, Analysis and Applications, Chemical Reviews, 111 (2011) 7355-7376.
2. M.J. Harsch, F. Benkwitz, A. Frost, B. Colonna-Ceccaldi, R.C. Gardner, J.-M. Salmon, New Precursor of 3-Mercaptohexan-1-ol in Grape Juice: Thiol-Forming Potential and Kinetics during Early Stages of Must Fermentation, Journal of Agricultural and Food Chemistry, 61 (2013) 3703-3713.
3. T. Allen, M. Herbst-Johnstone, M. Girault, P. Butler, G. Logan, S. Jouanneau, L. Nicolau, P.A. Kilmartin, Influence of Grape-Harvesting Steps on Varietal Thiol Aromas in Sauvignon blanc Wines, J. Agric. Food Chem., 59 (2011) 10641-10650.
4. D.L. Capone, D.W. Jeffery, Effects of Transporting and Processing Sauvignon blanc Grapes on 3-Mercaptohexan-1-ol Precursor Concentrations, J. Agric. Food Chem., 59 (2011) 4659-4667.
5. R. Larcher, G. Nicolini, L. Tonidandel, T. Román Villegas, M. Malacarne, B. Fedrizzi, Influence of oxygen availability during skin-contact maceration on the formation of precursors of 3-mercaptohexan-1-ol in Müller-Thurgau and Sauvignon Blanc grapes, Aust. J. Grape Wine Res., 19 (2013) 342-348.
6. A. Roland, J. Vialaret, A. Razungles, P. Rigou, R. Schneider, Evolution of S-Cysteinylated and S-Glutathionylated Thiol Precursors during Oxidation of Melon B. and Sauvignon blanc Musts, J. Agric. Food Chem., 58 (2010) 4406-4413.
7. A. Roland, R. Schneider, C.L. Guernevé, A. Razungles, F. Cavelier, Identification and quantification by LC-MS/MS of a new precursor of 3-mercaptohexan-1-ol (3MH) using stable isotope dilution assay: Elements for understanding the 3MH production in wine, Food Chem., 121 (2010) 847-855.