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Novel technological strategies to enhance tropical thiol precursors in winemaking by-products

Tomás Román Villegas [a], Loris Tonidandel [a], Bruno Fedrizzi [b], Roberto Larcher [a], Giorgio Nicolini [a,*]

[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

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ABSTRACT

Grape pomace is a winemaking by-product that can be used to extract oenological tannins. Recently, some grape skin tannins were shown to contain very high amounts of two polyfunctional thiol precursors (3-*S*-glutathionylhexan-1-ol, 3-*S*-cysteinylhexan-1-ol) whose free forms are responsible for appreciated tropical-like flavours. This study shows that an oxidative treatment (no SO_2) of white grape pomace and the presence of grape leaves and stems can increase the content of the above mentioned precursors. Moreover, it shows significant differences between Sauvignon Blanc, Gewuerztraminer and Mueller-Thurgau grape pomace for the 3-mercaptohexan-1-ol precursors and 4-*S*-cysteinyl-4-methylpentan-2-one. The grape cultivar is crucial, but the technological ability of enhancing the level of the volatile thiol precursors simply by treating the grape marc in different ways is a promising and powerful tool for the production of potentially flavouring tannins intended for food and beverage industry.

1. Introduction

The wine industry is producing a high amount of horticultural by-products, with an estimated value of 4 Mt/y worldwide (Corbin et al., 2015; FAO, 2015). Grape pomace is mainly constituted of berry skin and seeds, which are extremely rich in polyphenols and represent the starting material for the extraction of grape tannins (European Commission, 2003, 2004; Laufenberg, Kunz, & Nystroem, 2003; Teixeira et al., 2014).

Oenological tannins are a complex mixture in which polyphenolic compounds play a major role. They have been used in different fields like leather, adhesives, ore flotation, cements as well as pharmacy and medicine (Pizzi, 2008). Moreover, they are adjuvants commonly employed in the food and beverage industry for their ability to stabilise colour, enhance flavour complexity and modulate taste (Haslam, 2007; Vivas, 2000; Vivas, Bourgeois, Vitry, Glories, & deFreitas, 1996; Vivas & Glories, 1996; Vivas, Nonier, & Gaulejac, 2004; Vivas, Nonier, & Vivas de Gaulejac, 2003). Several biological sources can be used to extract these products and, among them, grape skin and seeds are gaining an increasing role given their ability to tailor the food and beverage sensory characteristics (Cliff, Stanich, Edwards, & Saucier, 2012; Sonni, Chinnici, Natali, & Riponi, 2011).

In the specific context of wine and beverages, tropical sensory notes are currently highly desired. The main molecules responsible for these aromas are polyfunctional thiols (3-mercaptohexan-1-ol, 3MH; 3-mercaptohexyl acetate, 3MHA; 4-mercapto-4-methylpentan-2-one, 4MMP) produced during fermentation from non-volatile *S*-glutathionyl and *S*-cysteinyl precursors or through other biosynthetic pathways involving H_2S and (*E*)-2-hexenal not completely elucidated (Fedrizzi, Pardon, Sefton, Elsey, & Jeffery, 2009; Harsch et al., 2013; Peyrot des Gachons, Tominaga, & Dubourdieu, 2002; Schneider, Charrier, Razungles, & Baumes, 2006; Tominaga, Peyrot des Gachons, & Dubourdieu, 1998; Winter, van der Westhuizen, Higgins, Curtin, & Ugliano, 2011).

We recently found that some commercial grape skin tannins can contain remarkable amounts of Cys-3MH and GSH-3MH (Larcher, Tonidandel, Nicolini, & Fedrizzi, 2013) and that their addition to juice prior to fermentation can increase the content of 3MH and 3MHA in wine (Larcher et al., 2015). This evidence prompted us to focus our attention on technological options to increase the level of the non-volatile thiol precursors in grape marc.

Some agronomical variables, *e.g.* type and timing of nitrogen fertilisation of vineyard or water and nitrogen deficit (Chone et al., 2006; Peyrot des Gachons et al., 2005), as well as varietal differences seem to affect the accumulation of thiol precursors (Larcher et al., 2013; Roland et al., 2011; Roland, Vialaret, Razungles, Rigou, & Schneider, 2010). To the best of our knowledge,

* Corresponding author.
E-mail address: giorgio.nicolini@fmach.it (G. Nicolini).

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the possibility of enhancing the level of the non-volatile thiol precursors by merely acting on grape pomace treatment has never been reported. This could be a powerful tool for the production of potentially flavouring tannins, with possible applications both in beverage production and food processing, as well as to add value to wine by-products. The possibility that these hydrophilic precursors could be rapidly hydrolysed in the mouth (Starkenmann et al., 2008) originating aromatic species, independently from a fermentation process, could represent a further interesting technological opportunity.

In the present work different technological strategies are presented. In particular, the effect of the supplementation of grape pomace with SO_2, grape leaves and stems on the concentration of Cys-3MH and GSH-3MH was evaluated. The former treatment employs a commonly utilised winemaking additive and represents a strategy easily extendable to several food and beverage industrial processes. The addition of leaves and stems in grape pomace mimics the outcome of the machine harvested grape where significant amount of matter other than grape is usually present.

2. Materials and methods

2.1. Chemicals

Ultrapure water was produced with an Arium Pro UV DI Ultrapure Water System (Sartorius, Göttingen, Germany). Liquid chromatography-mass spectrometry grade formic acid (FA) and acetonitrile (ACN), ⩾97% potassium metabisulfite, ⩾98% L-ascorbic acid, ⩾98% reduced GSH, anhydrous ⩾99% dimethyl dicarbonate and ⩾99.9% methanol were supplied by Sigma-Aldrich (Milan, Italy). Buchem B.V. (Apeldoorn, the Netherlands) supplied d_3-3-*S*-cysteinylhexan-1-ol (d_3-Cys-3MH) and d_3-3-*S*-glutathionylhexan-1-ol (d_3-GSH-3MH), along with unlabelled 3-*S*-cysteinylhexan-1-ol (Cys-3MH) and 3-*S*-glutathionylhexan-1-ol (GSH-3MH). 4-*S*-cysteinyl-4-methylpentan-2-one (Cys-4MMP) and 4-*S*-glutathionyl-4-methylpentan-2-one (GSH-4MMP) were synthesised at the School of Chemical Sciences of the Auckland University following the protocols reported in the literature (Fedrizzi et al., 2009; Tominaga, Peyrot des Gachons, & Dubourdieu, 1998).

2.2. Sample preparation

2.2.1. General

Two experiments, named "SO_2" and "Stems/Leaves", have been carried out at the Experimental Winery of the Edmund Mach Foundation, San Michele all'Adige (Italy) during the vintage 2013.

2.2.2. SO_2 experiment

Pomace samples of Mueller-Thurgau (MT; N. = 10; juice composition, mean values: total soluble solids 16.6 °Bx, pH 3.18, titratable acidity 6.0 g/L as tartaric acid), Gewurztraminer (TR; 24; 23.5, 3.40, 4.1) and Sauvignon Blanc (SB; 12; 22.0, 2.94, 8.3) were obtained after crushing-destemming (Ares 15, OMAC s.r.l., Corridonia, MC, Italy) and pressing (3.5 bar; 20L Hydropress, Spiedel GmbH., Ofterdingen, Germany) grapes harvested according to the industrial plans of the commercial wineries of the region, with no SO_2. Each pomace sample, made up of 500 g, was divided into 2 equal aliquots named "SO_2" and "Control", respectively. To the first aliquot, Milli-Q water (50 ml) containing 80 mg of SO_2 (as potassium metabisulfite) was added. The second one was supplemented with 50 ml Milli-Q water and 25 mg NaN_3 to drastically reduce microbial activity. After a 5-h maceration at room temperature (16°–23 °C), 100 g sample was ground with methanol (80 ml) to stop enzymatic reactions and kept at −20 °C until analysis.

2.2.3. Stems/leaves experiment

Non mono-varietal white grape pomace (N = 16, ~30 kg each), sampled directly from the hopper at the end of the pressing cycle at industrial ripeness of juice (22.3 ± 1.1 °Bx), were collected from different wineries in Trentino (Italy). Each sample was divided into 3 aliquots: one supplemented with 3 grape leaves per 100 g pomace, another with 3 stems per 100 g pomace and the last aliquot without any supplementation (Control). Finally, the samples were ground and stored as above until analysis. The amount of leaves and stems adopted was merely chosen to verify, on a qualitative level, the effect of the treatment in the thiol precursors production.

Before analysis, the sample was remixed and a 15 g aliquot was taken and spiked with an H_2O:CH_3OH (1:1; v:v; 15 ml) solution. Then, it was supplemented with d_3-Cys-3MH (1.57 μg) and d_3-GSH-3MH (1.64 μg) as labelled internal standards, stirred (10 min), centrifuged (4000 rpm × 5 min; Centrifuge 4226, ALC International s.r.l., Milan, Italy) and finally the supernatant was filtered (0.22 μm) and injected.

2.3. Chemical and statistical analysis

LC-MS/MS quantification of thiol precursors was carried out using an UPLC Acquity coupled with a Xevo TQ MS mass spectrometer (Waters Corporation, Milford, USA). A 5 μl sample was injected onto an Acquity UPLC HSS T3 C18 column (1.8 μm film thickness, 2.1 mm × 100 mm; Waters). Flow rate was set at 0.45 ml/min and column temperature at 40 °C. MS isotopic dilution analysis was performed in positive ion mode (capillary voltage, 2.5 kV), with argon (0.20 ml/min) and nitrogen (1000 L/h) as collision and desolvation gas, respectively. Other characteristics of the method are specified in a previous work (Larcher et al., 2013).

Box plots and statistical tests were carried out using STATISTICA v. 8.0 (StatSoft Inc., Tulsa, OK).

3. Results and discussion

3.1. SO_2 experiment

The thiol precursors measured in the 92 grape pomace samples determined according to the protocol discussed above, ranged between 23 and 3478 μg/kg and 55–4432 μg/kg for the Cys-3MH

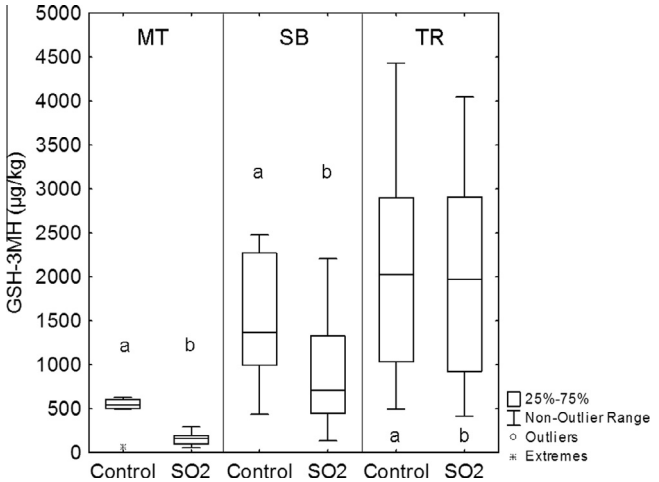

Fig. 1. 3-*S*-glutathionylhexan-1-ol (GSH-3MH) content in Mueller-Thurgau (MT), Sauvignon Blanc (SB) and Gewuerztraminer (TR) grape pomace processed with (SO_2) or without (Control) supplementation of 320 mg/kg SO_2. Box plots with different letters are statistically different (Sign test, $p < 0.05$).