CLARIFYING AGENTS AND 3-SULFANYLHEXANOL PRECURSORS IN GRAPE JUICE

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ABSTRACT

We evaluated the impact of a number of clarifying agents on the concentration of S-3-(hexan-1-ol)-L-cysteine (Cys-3SH) and S-3-(hexan-1-ol)-L-glutathione (GSH-3SH). 19 clear grape juices were spiked with a grape skin tannin rich in Cys-3SH and GSH-3SH. Juices were then treated with Na-bentonite, PVPP or charcoal (1 g/L) and cold settled. The concentration of precursors was measured and compared to the corresponding untreated control juices in the devatted samples. Cys-3SH and GSH-3SH were analysed using UHPLC-MS/MS and accuracy was guaranteed with deuterated internal standards. Only charcoal caused a statistically significant depletion of both precursors, quantitatively limited even at the highest dose adopted. Technologically, the clarifiers used in juice affected the thiol precursors in a marginal manner.

Keywords: bentonite, charcoal, grape juice, polyvinylpolypyrrolidone, varietal thiols

1. INTRODUCTION

S-3-(hexan-1-ol)-l-cysteine (Cys-3SH) and S-3-(hexan-1-ol)-l-glutathione (GSH-3SH) are precursors, present in grapes and/or formed in juice (TOMINAGA and DUBOURDIEU, 2000; PEYROT DES GACHONS *et al*., 2002; SCHNEIDER *et al*., 2006; FEDRIZZI *et al*., 2009; ROLAND *et al*., 2011a), of 3-sulfanylhexanol (3SH), responsible - together with its acetate - for the tropical and grapefruit-like fruity notes produced during fermentation by some yeast strains having lyase activity (RONCORONI *et al*., 2011; WINTER *et al*., 2011). The grape variety, as well as the processing conditions of grape, pomace and juice, are very important in saving/producing a high level of precursors (ROLAND *et al*., 2011a; CERRETI *et al*., 2015; ROMÁN VILLEGAS *et al*. 2016). Technologically speaking, for example, 3SH precursors increase with longer skin-contact and stronger pressing conditions (MATTIVI *et al*., 2012) and GSH-3SH in particular increases when oxidative pre-fermentative maceration is adopted (LARCHER *et al*., 2013a). Nevertheless, the effects of the main clarifying agents on the content of the precursors cited are little known to date. For this reason, the aim of the experiment reported in this paper was to investigate whether certain common clarifiers used in juice can modify the concentration of Cys-3SH and GSH-3SH.

2. MATERIALS AND METHODS

2.1. Juice preparation

Nineteen lots of must (20 L) were produced from sound white and red grapes coming from different varieties and plots in Trentino (Northern Italy), selected in order to include a wide compositional variability. Grape lots (300 Kg) were destemmed, crushed and pressed (3.5 bar; press mod. UP600, Willmes, Lorsch, Germany) on a semi-industrial scale at the Edmund Mach Foundation experimental winery (San Michele all'Adige, Italy). To ensure a high concentration of thiol precursors, only the juice fraction over 65% w/v yield was used in the experiment (MAGGU *et al*., 2007; ALLEN *et al*., 2011; ROLLAND *et al*., 2011b). Moreover, the juices were indirectly enriched randomly with 500-1000 mg/L of grape skin tannin containing 224.2 mg/kg GSH-3SH and 25.5 mg/kg Cys-3SH, quantified according to LARCHER *et al*., (2013b), and supplemented with a volume of 15-25% of Sauvignon Blanc juice, a variety well-known for its richness in thiol precursors (CAPONE *et al.,* 2010; LARCHER *et al*., 2013a). After sulfiting (20 mg/L SO_2), all juices were cold settled (< 20 nephelometric turbidity units), well beyond normal winemaking practice, in order to minimise the effect of solids suspended in the turbid juice. Clear juices were then devatted, divided into 4 fractions of 5 L each and supplemented with activated Na bentonite (Pentagel, 1 g/L; Perdomini-IOC S.p.A., S. Martino Buon Albergo, Italy), charcoal (Eno Anticromos, 1 g/L; Dal Cin S.p.A., Concorezzo, Italy) or polyvinylpolypyrrolidone (PVPP V, 1 g/L; Perdomini-IOC) in comparison with the unspiked fraction respectively. After treatment, all samples were cold settled again for 48h at 4°C.

2.2. Sampling

The settled juice was sampled (25 mL), supplemented with methanol (25 mL, -20°C) and stored at -20°C until analysis. The methanol solution was spiked with d_3-GSH-3SH and d_3-Cys-3SH as labelled internal standards and filtered through a 0.22 μm filter (Millex-GV, Millipore, Ireland) before analysis.

2.3. Chemical analysis

The juice composition was analysed using a WineScan FT 120 Type 77310 (Foss, Hillerød, Denmark), accurately aligned according to the official methods (OIV 2012).
An UPLC Acquity system coupled with a Xevo TQ MS mass spectrometer (Waters Corporation, Milford, USA) was used for LC-MS/MS quantification of thiol precursors. A 5 μL sample was injected into an Acquity UPLC HSS T3 C18 column (1.8 μm film thickness, 2.1 mm × 100 mm; Waters) set with a flow rate of 0.45 mL/min and a temperature of 40°C. MS isotopic dilution analysis was performed in positive ion mode (capillary voltage, 2.5 kV), using argon (0.20 mL/min) and nitrogen (1,000 L/h) as collision and desolvation gas respectively. Other characteristics of the method are specified in LARCHER *et al.* (2013a).

2.4. Statistical analysis

Anova (main effects: juice, clarifier) and Tukey's HSD test were carried out using STATISTICA v. 8.0 (StatSoft Inc., Tulsa, OK).

3. RESULTS AND CONCLUSIONS

The clarifiers were chosen because they are extensively used in winemaking during prefermentation manipulation of white grape must, due to their depletion features in relation to specific classes of compounds (bentonite vs. proteins; PVPP vs. polyphenols) or to their high but non-selective adsorption capacity (charcoal). To our knowledge, there are no reports that specifically link fining agents and thiol precursor content, while their depletion capacity has been previously reported in relation to free and bound primary aromas (MOIO *et al.*, 2004) and other odour active compounds in juice (LAMBRI *et al.*, 2010).
The juices were chemically characterised by their base composition (mean ± st. dev.; min - max) for total soluble solids (21.2±2.0 °Brix; 18.6-26.0), pH (3.23±0.11; 3.01-3.45), titratable acidity (6.63±1.23 g/L; 4.70-10.00), tartaric acid (5.76±0.69 g/L; 4.93-7.62), malic acid (3.25±0.76 g/L; 1.93-4.68), potassium (1348±158 mg/L; 1086-1618). These data highlight the considerable compositional variability used to ensure the robustness of the results, since the grape cultivar and ripeness not only affect the precursor content (KOBAYASHI *et al.*, 2010; CERRETI *et al.*, 2015) but also influence either the composition (Pirie and Mullins, 1977; POCOCK *et al.*, 2000) or the haze (MESQUITA *et al.*, 2001) of the most usual target molecules for these clarifiers and hence the clarifying activity.
The ranges obtained for GSH-3SH (min-max: 240 - 564 μg/L) and Cys-3SH (36,5 - 244 μg/L) in the control juices match the literature (PEÑA GALLEGO *et al.*, 2012; LARCHER *et al.*, 2013a;). Comparison of the results of the corresponding control juices and treated samples showed that bentonite and PVPP had a limited and not statistically significant effect (Table 1) on the concentration of GSH-3SH and Cys-3SH. On the contrary, charcoal treatment significantly reduced (p<0.05) the two thiol precursors, however this reduction was limited, being roughly 20% for GSH-3SH and 10% for Cys-3SH.
The significance of these results is not limited to winemaking, but could also be of interest for the grape juice industry, where there is the possibility of using hybrid varieties, resistant to mold diseases and consequently with lower operating costs. Precursors are also present in their juices (LARCHER *et al.*, 2014), and the release of 3SH through specific commercial enzymes could contribute to overall aroma.

Table 1: Thiol precursors in juice in relation to the clarifying agent used. (Values with the same letter are not statistically different in Tukey's HSD test, $p<0.05$; n.s. = non significant).

	GSH-3SH (μg/L)			Cys-3SH (μg/L)		
Treatment	**Mean (n=19)**	**S.D.**	**sign.**	**Mean (n=19)**	**S.D.**	**sign.**
Control	344	73	a	80	53	a
Bentonite (1 g/L)	336	75	a	79	53	a
Charcoal (1 g/L)	276	66	b	73	46	b
PVPP (1 g/L)	344	74	a	80	53	a

In conclusion, of the clarifying agents used in this experiment, only charcoal proved able to significantly reduce 3-sulfanylhexanol precursors in juice. Nevertheless, in the light of the usually lower doses of these products adopted for juice in modern white winemaking, the low conversion ratios of the precursors to the corresponding free thiols (ROLAND *et al*., 2011a), and the limited percentage changes observed in this experiment, it can be deduced that the clarifying agents used affect the content of thiol precursors in a technologically and sensorially negligible manner, despite the low sensory threshold of the relative derivatives in free and acetate form.

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