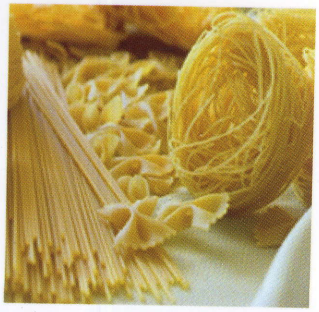

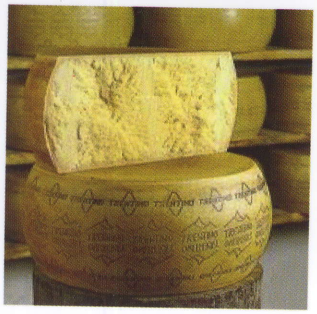

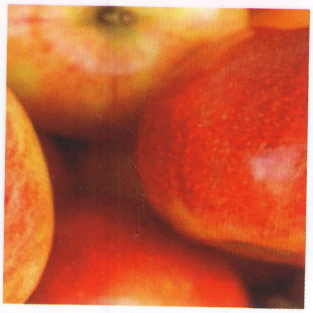

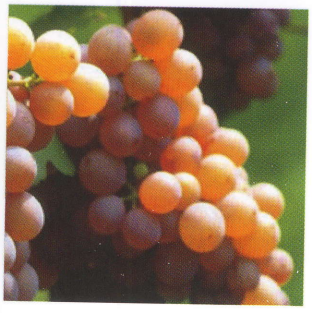

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BOOK OF ABSTRACTS

3rd MS Food Day

2013 October 9-11
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BOOK OF ABSTRACTS

Franco Biasioli
Editor

Fondazione Edmund Mach - 2013

P.03 - First identification of S-3-glutathionylhexanal in Sauvignon Blanc grapes using LC-MS/MS experiments

Roberto Larcher[1*], Loris Tonidandel[1], Giorgio Nicolini[1], Maria Stella Grando[1], Paula Moreno-Sanz[1], Bruno Fedrizzi[2]

1 Fondazione Edmund Mach (FEM), via E. Mach 1, 38010 San Michele all'Adige (TN), Italy
2 Wine Science Programme, School of Chemical Sciences, The University of Auckland, Private Bag 92019, Auckland, New Zealand
* *roberto.larcher@fmach.it*

Summary: Varietal thiols with their extremely low threshold and the high appreciation towards the perfumes they impart are one of the most widely studied classes of molecules in the wine. Particular emphasis was also placed on their biogenesis and so far three pathways were identified. Researchers working in the field have also tried to understand how winemaking could impact the level of their precursors suggesting the possibility for a de novo synthesis. This communication reports the first identification of S-3-glutathionylhexanal in grape samples. This evidence confirms the possibility for a de novo synthesis of these molecules that could then be metabolised during fermentation.

Keywords: Varietal thiols; S-glutathionylated precursors; S-gluthathionylhexanal

Introduction

Varietal thiols are certainly a hot topic in wine chemistry. The impact that these molecules impart to wines is extremely appreciated. Since their discovery in grape and wine in 1993 a vast number of publication on this matter have followed. Currently we can account for 2 non-volatile precursors (S-glutathionylated and S-cysteinylated) and a direct conjugation between H_2S and (E)-2-hexenal. As far as concerns the first two precursors a particular emphasis was placed on the possibility of a technological induction in the biosynthesis of these molecules. The natural availability of glutathione (GSH) in grape and the production of (E)-2-hexenal at crushing corroborated the idea that a tailored technological approach could increase the level of these precursors in grape juice, potentially enhancing the level of free thiols in finished wine.
Up to day three molecules in the de novo synthetic pathway have been identified. Nonetheless the product of the first crucial step, the conjugation between GSH and (E)-2-hexenal was never identified in grape samples.
This work reports the first identification of this molecule in grape sample samples by means of a synergistic approach combining synthetic strategies and MS-based structural identification.

Experimental

About 40 g of berries were randomly sampled from several bunches of Sauvignon Blanc during the 2012 harvest. Any berry damages were avoided and the pedicel was cut. The berries were immediately stored in liquid nitrogen and conserved at -80°C until analysis. They were then ground using an A11 basic analytical mill (IKA) keeping them in liquid nitrogen during the whole process. 2 g of the resulting fine powder was weighted (SBC 31 analytical balance; Scaltec) into a 50 mL falcon and added of 8 mL of extraction solution (80% methanol, 20% milliQ water, and the deuterated standards of Cys-3-MH and Glut-3-MH). The mixture was then mixed for 10 min and centrifuged for 5 min at 4000 rpm at room temperature. Finally, the solution was filtered (0.22 μm) and 1.5 mL was transferred into a 2 mL glass vial for the LC-MSMS analysis.
Identification of the S-3-glutathionylhexanal was performed using an Acquity UPLC Waters equipped with a Xevo TQ MS mass spectrometer (Waters Corporation, Milford, US), injecting

5 µL on an Acquity HSS T3 C18 column (1.8 μm film thickness, 2.1 mm × 100 mm; Waters). Flow rate was set at 0.45 mL/min. Experimental conditions were based upon Larcher and co-workers (2013) [1]. Chromatographic separation was obtained using the following gradient (water as eluent A and ACN as B, both added of 0.1% formic acid): B was held at 5% for 2 minutes, raised to 100% in 5 minutes, then held at 100% for 1 minute, and back to 5% in 0.01 minutes. Column reconditioning was performed holding B at 5% per 2 minutes before the next injection. Analysis was performed in ESI+ (capillary voltage, 2.5 kV) and with argon (0.20 mL/min; gas) and nitrogen (1000 L/h;) as collision and desolvation gas, respectively, used deuterated internal standards. Cone voltage potential was 22 V, collision energy 14 eV.

Results

Figure 1 shows the PI mass spectra of the synthetic and the natural S-3-glutathionylhexanal, with fragments at m/z 406, 388, 331, 259 and 162 belonging to the pseudo molecular ion $[M+H]^+$, the loss of 18 $[M+H - H_2O]^+$, the loss of 76 $[M+H - GlyOH]^+$, the loss of 147 $[M+H - C_5H_{11}O_3N_2]^+$, the loss of hexanal moiety from ion at m/z 259 $[M+H - C_5H_{11}O_3N_2 - Hexenal]^+$. The samples were quantified in MRM mode using the following m/z transition for quantification 406→259 [2]. The methanolic solution kept at 20°C showed since the preparation of the samples a steady de-novo formation trend (1.5 μg/Kg per hour) of the S-3-glutathionylhexanal for all the observing period (150 hour), rising up to 230 μg/Kg. This results could suggest that the enzyme glutathione S-transferase involved in the grapevine biosynthetic pathway for the formation of 3-S-glutathionyl mercaptohexan-1-ol and 3-S-cysteinyl mercaptohexan-1-ol from glutathione and (E)-2-hexenal, is not completely inhibited by the methanolic medium, while the subsequent reductase activity is, reasonably, partially or completely blocked [3].

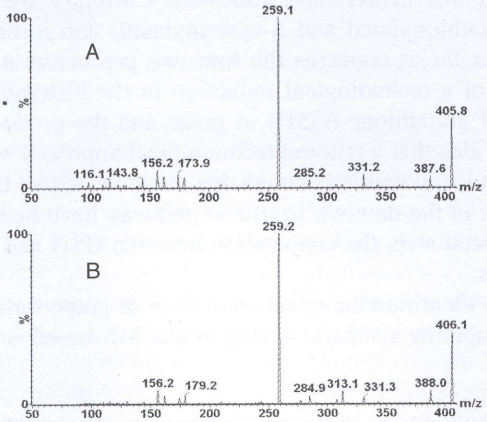

Figure 1. Product ion mass spectra of S-3-glutathionylhexanal in synthetic solution (A) and natural juice (B).

Conclusions

This communication proves for the first time the presence of S-3-glutathionylhexanal in juice giving further evidence to the correctness of the biosynthetic pathway proposed for the formation of the varietal precursor of 3-mercaptohexan-1-ol.

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