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Changes in chemical and sensory properties of Amarone wine produced by *Penicillium* infected grapes

Giacomo Zapparoli[a], Marilinda Lorenzini[a], Emanuele Tosi[b], Michela Azzolini[a], Davide Slaghenaufi[a], Maurizio Ugliano[a], Barbara Simonato[a,*]

[a] Dipartimento di Biotecnologie, Università degli Studi di Verona, Verona, Italy
[b] Centro per la Sperimentazione in Vitivinicoltura, Provincia di Verona, San Pietro In Cariano, Verona, Italy

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ABSTRACT

There is very little information on effects of *Penicillium* on aroma of passito wine. This study analyzed chemical composition and sensory properties of Amarone wines produced from withered grapes artificially contaminated by *P. expansum* or *P. crustosum*. Changes in properties of the two wines were evident by comparing wines obtained from healthy and *Botrytis cinerea* infected grapes used as controls. *Penicillium* infection affected primary and volatile composition of Amarone wine. Sensory profiles of these wines, obtained by descriptive analysis, resulted in clear differences in the wines between themselves and the control wines. Partial least square regression analysis explained only partially the relationship between molecules and sensory descriptors, and showed the existence of complex interactions of compounds mainly involved in specific aroma attributes. GC-olfactive analysis showed a greater number of odour regions in *P. crustosum* wine compared to control wines. Useful insight was provided into understanding how *Penicillium* rotten grapes affect Amarone wine properties.

1. Introduction

Penicillium is a pathogenic agent of wine grapes and is one of the most common genera isolated in the vineyard (Rousseaux, Diguta, Radoï-Matei, Alexandre, & Guilloux-Bénatier, 2014). Studies have focused on *Penicillium*, which is of particular interest in terms of production of mycotoxins and off-flavour in wine (La Guerche, Dauphin, Pons, Blancard, & Darriet, 2006; La Guerche, De Senneville, Blancard, & Darriet, 2007; Morales-Valle, Silva, Paterson, Venacio, & Lima, 2011).

Penicillium infection has an impact on the sensory quality of wine. Together with other fungi occurring on rotten grapes (e.g. *Botrytis*, *Aspergillus*, *Rhizopus* and *Coniothyrium*) this fungus is responsible for the production of mushroom- and earthy-smelling compounds that may cause defects in wine (La Guerche et al., 2006, 2007).

Penicillium has been detected in grapes used for passito wine production during post-harvest dehydration (withering) (Lorenzini, Azzolini, Tosi, & Zapparoli, 2013; Lorenzini, Cappello, Logrieco, & Zapparoli, 2016; Torelli, Firr, Locci, & Gobbi, 2006). The most contaminant species of this genus are *P. expanusm* and *P. crustosum* (Lorenzini, Cappello, et al., 2016). Incidence of their infection depends upon culture management, seasonal conditions and the withering process. The infection incidence may range from less than 1 to 15% of all the withered grapes stored in fruit-drying room, while highly contaminated bunches may have up to 40% of infected berries (data not published). Although these species are very frequent in withered grapes, there is still very little information on their potential role in passito wine production. Our proteomic investigation revealed that *Penicillium* infection affects protein patterns in withered grapes and identified potential fungal markers of *Penicillium* presence in grapes (Lorenzini, Mainente, Zapparoli, Cecconi, & Simonato, 2016). Similar data were obtained for *Botrytis* infection in withered grapes (Lorenzini et al., 2015) which impact as noble rot on aroma. The sensory quality of passito wines obtained from noble-rotten grapes has also been investigated (Fedrizzi et al., 2011; Tosi et al., 2013). Conversely, the effects of grape contamination by *Penicillium* on overall quality of passito wine are still unknown.

The aim of this study was to assess the main effects of two *Penicillium* species, *P. expansum* and *P. crustosum*, on aroma of Amarone wine, a red dry passito wine of considerable economic importance. This is the first comprehensive evaluation of the chemical composition and sensory properties of Amarone wine produced from withered grapes infected by *Penicillium*.

* Corresponding author.
E-mail address: barbara.simonato@univr.it (B. Simonato).

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2. Materials and methods

2.1. Strains

Two strains, *P. expansum* A3 and *P. crustosum* C2, out of 50 isolates collected from withered grapes during the 2012–16 growing season were selected for the present study. These two strains displayed high disease index assayed on withered grapes in fruit-drying room (data not shown). The pathogenicity of these two strains was suitable to infect the grapes used for the present study. *Botrytis cinerea* B2 was a selected strain used in previous investigations (Lorenzini et al., 2013, 2015).

2.2. Grape infection and microvinification

To evaluate the effect of *Penicillium* on Amarone wine composition, an infection assay of grapes of Corvina variety was carried out in the winery of Centro per la Sperimentazione in Vitivinicoltura, Provincia di Verona (Italy). The protocol of fungal suspension preparation for each strain (*P. crustosum* C2 and *P. expansum* A3) and infection of grapes wounded by piercing the berries were carried out as previously described (Tosi et al., 2013). Briefly, about 580 kg of fresh grape bunches were homogeneously divided in four lots (145 kg each) in order to produce four types of experimental wines indicated as C2 and A3 wine (*Penicillium* wines) from infected grapes by *P. crustosum* C2 and *P. expansum* A3, respectively, botrytized (B) wine from infected grapes by *B. cinerea* B2 and healthy (H) wine from uninfected grapes. In addition to H wine, B wine was used as the control since noble rot effects on aroma of Amarone wine were previously reported (Fedrizzi et al., 2011; Tosi et al., 2012).

The grapes were placed in plastic boxes, previously washed with hot water and then alcoholic solution (ethanol: water 1:2 v/v). In order to avoid cross-contaminations, the boxes were kept in three separated locations within natural fruit-drying room without any control of temperature and relative humidity according to the traditional procedure of the Valpolicella area (Italy). The evolution of infection was monitored by visual inspection. Estimation of infection level on grapes was carried out by determining the frequency of berries with typical symptoms by *Penicillium* and *Botrytis* contamination in a representative grape bunches for each box. The presence of *Penicillium* strains, selected for this study, on infected grapes was ascertained by the identification of 30 representative isolates of each species using culture and molecular analysis as previously described (Lorenzini, Cappello, et al., 2016). At the end of withering, the incidence of symptomatic berries due to *Penicillium* infection on grapes infected by *P. expansum* A3 or *P. crustosum* C2 strains were approximately 25 and 23%, respectively. Grapes infected by *B. cinerea* B2 strain displayed about 29% of berries with symptoms of *Botrytis* contamination.

The weight loss of grapes was monitored every 7–10 days. The end of withering was established when the weight loss was about 35–40% w/w. The withering kinetic resulted similar in all four trials (the final weight loss of grapes was 37.3 ± 3.0, 39.1 ± 2.6, 36.9 ± 2.8, 37.4 ± 2.8 w/w for H, B, A3 and C2 trial, respectively). Each grape bulk was crashed separately dividing the must and pomace in three fractions (microvinifications in triplicate) of 20 l and alcoholic fermentation was driven by *S. cerevisiae* EC1118 (Lallemand Inc, Montréal, Canada). The trials were located in a winery room without controlled temperature and after the fermentation the resulting wines were devatted and decanted at 4 °C for 3 days, then stabilized adding 50 mg/l SO_2 before the analysis. Wines were kept in an appropriate winery room for the aging and after three years were submitted to chemical and sensory analysis.

2.3. Chemical analysis of wine

Ethanol was analyzed by NIR spectroscopy using Alcolyzer Wine apparatus (Anton Paar GmbH, Graz, Austria). Reducing sugars, titratable acidity (as tartaric acid) and volatile acidity (as acetic acid) were determined by titration according to standard analysis methods. Glycerol, L-malic acid and L-lactic acid was enzymatically determined (F. Hoffmann-La Roche AG, Basel, Switzerland). Total polyphenols (as gallic acid) and total anthocyanins (as malvidine 3-glucoside) were determined by using Folin-Ciocalteu method and pH-differential method, respectively. Wine colour parameters were determined spectrally by measuring absorbance at 420, 520 and 620 nm.

2.4. Analysis of volatile compounds

Volatile compounds, such as ethyl acetate, acetaldehyde, 1-butanol, 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol and 3-methyl-1-butanol were analyzed by a Vega series 6000 gas chromatograph (Carlo Erba Instruments, Milan, Italy) equipped with a 200 cm × 2 mm i.d. glass packed column filled with 80/20 Carbopack B AW/6.6% and PEG 20M (Supelco, Bellefonte, PA) and with a Flame Ionization Detector (FID). Column temperature varied from 80 to 180 °C with a rate of 4 °C/min; injector and detector temperature was 200 °C and carrier gas (N_2) flow rate was 20 ml/min. The gas chromatograph was connected to an HS 250 autosampler (Carlo Erba Instruments). Two µl of wine sample spiked with 100 mg/l 2-butanol (as internal standard) were used for each analysis.

Solid Phase Extraction (SPE), using 1 g ENV$^+$ cartridges (Isolute, IST Ltd., Mid Glamorgan, UK), was performed to quantitatively extract the volatile compounds; the extraction was carried out one time for each sample and subsequently the extract was injected into a gas chromatography–mass spectrometry (GC–MS). The SPE method was performed on an automated solid phase extraction apparatus (Aspec XL Gilson Inc., Middleton, USA). The cartridges were sequentially conditioned with methanol (10 ml) and MilliQ water (10 ml). A total of 76 ml of wine sample diluted 1:4 v/v, with distilled water and added with 1-heptanol as internal standard (500 µg/l), were loaded onto the cartridge. The cartridge was then rinsed with 10 ml of distilled water. The residual was washed with 10 ml of distilled water. The free volatile compounds were eluted with 9 ml of dichloromethane. The solution was dried with Na_2SO_4 and concentrated to 0.4 ml by a gentle nitrogen flow.

GC–MS analysis was performed with a 6890 N Network GC System coupled with a 5978B inert XL EI/CI MS (Agilent Technologies, Milan, Italy), equipped with a HP-WAX Bonded PEG fused silica capillary column (60 m × 320 µm i.d. × 0.25 µm film thickness; Agilent Technologies). MS conditions were: electron impact energy 70 eV and MS source temperature 230 °C. The temperature of transfer line and GC injector temperature were 200 and 250 °C respectively and helium was used as carrier (flow: 1.5 ml/min). Column temperature program was: 50 °C (4 min), 4 °C/min to 240 °C, 240 °C (16 min).

Identification of volatile compounds was achieved by means of pure reference standard (Sigma-Aldrich, St. Louis, MO, U.S.) co-injections; when the authentic standards were not available the identification was based on the comparison with the spectral data of the NIST library.

Quantitative analysis was performed by total ion chromatograph using the response factors calculated for each compound by calibration curves in a hydroalcoholic solution, containing 12% v/v ethanol with 5 g/l tartaric acid. For compounds for which commercial standards were not available, the response factors of compounds with similar chemical structures were utilized.

The odour activity values (OAV) were calculated as the ratio between the measured concentration of a substance in the wine and its odour threshold, when available.

2.5. Gas-chromatography analysis with olfactive detection (GC-O)

GC-O analyses were performed on a HP-7890 (Agilent Technologies, Palo Alto, USA) gas chromatographer equipped with an olfactometer port ODP-3 (Gerstel, Müllheim/Ruhr, Germany). An aliquot of 2 µl of