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Fungicide residues affect the sensory properties and flavonoid composition of red wine

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Chemical compounds studied in this article:
Metrafenone (PubChem CID: 6451057)
boscalid (PubChem CID: 213013)
kresoxim-methyl (PubChem CID: 6112114)
fenhexamid (PubChem CID: 213031)
mepanipyrim (PubChem CID: 86296)

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ABSTRACT

The influence of four fungicide treatments [viz., metrafenone, boscalid + kresoxim-methyl, fenhexamid and mepanipyrim, which are typically used to control downy mildew (*Plasmopara viticola*) and grey mould (*Botrytis cinerea*)] on the composition of Tempranillo red wines was assessed by examining changes in phenolic composition, colour and aroma profile in wines from pesticide-treated grapes in relation to control wines made from untreated grapes. The results were also compared with those for wine of a previous vintage in order to ascertain whether fungicide-related changes were comparable to vintage-related changes. Only the boscalid + kresoxim-methyl treatment led to significant differences in wine of the 2013 vintage; thus, it increased the contents in monomeric anthocyanins (58%) and flavan-3-ols (36%), and also colour lightness (20%), but decreased the contribution of the ripe (42%) and fresh fruits (59%) odorant series. These results seemingly confirm that the presence of boscalid + kresoxim-methyl residues in must impairs the sensory quality of the resulting wine by diminishing its brightness and aroma. Differences varied markedly between years, which suggests that the course of the fermentation process is influenced not only by the composition of the grapes but also by the presence of the fungicide.

1. Introduction

Vitis vinifera cv. Tempranillo is the most characteristic variety of the Qualified Designation of Origin “Rioja” in Spain. One of the main difficulties in growing grapes for wine arises from the adverse effects of fungi (e.g., botrytis, powdery mildew, downy mildew). Also, the presence of fungicide residues in red grapes may alter their phenolic composition and/or the extraction of phenols during the winemaking process, colour, the biosynthesis of aroma compounds, and also the phenolic composition during storage and aging. Phenolic compounds are the main source of wine colour and astringency, the latter of which is mainly due to tannins (Quijada-Morín et al., 2012). In addition, phenolic compounds possess antioxidant and free radical scavenging properties (Mulero et al., 2010).

The effects of fungicides on the colour and phenolic profile of red wines remains poorly studied owing to their complexity relative to the effects on the volatile profile, for example. Fernández et al., (2001) examined the influence of azoxystrobin, cyprodinil, fludioxonil, kresoxim-methyl, pyrimethanil and quinoxyfen on the final colour of wines from treated grapes and found significant differences from the control wine in all target parameters except total anthocyanins and ortho-diphenols, the differences being especially marked in wines obtained from pyrimethanil-treated grapes. Oliva et al. (2009) found the phenolic composition of Monastrell red wines to be altered by the presence of residues of famoxadone, fenhexamid, fluquinconazole, kresoxim-methyl, quinoxyfen and trifloxystrobin applied in vineyard; also, they found the absolute values of antioxidant activity to be higher in the treated wines. Briz-Cid et al. (2014) assessed the effects of two antifungal treatments (viz., metrafenone and boscalid + kresoxim-methyl) applied in vineyard in accordance with Good Agricultural Practices (GAP) on the colour and phenolic profile of red wines from Tempranillo and Graciano grapes. Irrespective of variety, the wines from grapes treated with boscalid + kresoxim-methyl were slightly shaded and exhibited greater changes in phenolic profile than the control wines. Similarly, Briz-Cid et al. (2015) studied the effects on wine colour of the presence of residues of the fungicides fenhexamid and mepanipyrim in must at concentrations equivalent to their Maximum Residue Limits (MRLs) in grapes in order to mimic Critical Agricultural Practices (CAP). The differences between treated and control wines were greater than those observed under GAP conditions, which suggested that the

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effect of fungicides on wine colour was concentration-dependent. Monomeric anthocyanins and flavan-3-ol monomers were the most markedly affected compounds. Recently, Mulero et al. (2015) examined the impact of six fungicides (viz., fenhexamid, kresoxim-methyl, fluquinconazole, famoxadone, quinoxyfen and trifloxystrobin) applied under CAP conditions on phenolic composition and antioxidant activity in Monastrell red wines. The wines made from quinoxyfen-treated grapes exhibited increased phenol contents relative to the control wines; by contrast, those made from trifloxystrobin-treated grapes had the lowest phenol concentrations. Antioxidant activity was not altered by quinoxyfen, fluquinconazole or famoxadone, but was decreased by the other three pesticides. In summary, existing studies have exposed the individual influence of anti-mildew, anti-oidium and anti-botrytis fungicides on the phenolic profile of wine.

Because wine is a beverage consumed by pleasure, its colour and aroma are essential with a view to its positioning as a good purchase option for consumers. Improving our understanding of the impact of fungicides on wine quality is therefore of paramount importance. The primary aim of this work was to assess the effect of fungicides on the colour and aroma profile (especially the phenolic profile) of Tempranillo red wines by comparing the results for wines of two different vintages (2012 and 2013). This work is related to a previous study where we examined the influence of the same fungicides on the volatile profile of the wines (Noguerol-Pato et al., 2016) by comparing fungicide dissipation during winemaking and the effects of the pesticides on fermentation.

2. Experimental

2.1. Fungicide treatments

Fungicide treatments were applied in 2013 to the same experimental vineyard previously used by Briz-Cid et al. (2014, 2015). The vineyard, which is located in Aldeanueva de Ebro (La Rioja, NE Spain), was split into three experimental plots (A–C) containing 6 vine rows each. Plot A was left untreated. Plots B and C were treated with the phytosanitary products Collis® and Vivando®, respectively, both from BASF Crop Protection Spain (Barcelona, Spain); the former contains 20% w/v boscalid + 10% w/v kresoxim-methyl and the latter 50% w/v metrafenone. Both treatments were applied in accordance with Good Agricultural Practices (i.e., by using the manufacturer's recommended dose and observing/the pre-harvest time in vines). Grapes from each plot were harvested separately, and only those from the two central rows were used for vinification in order to avoid cross contamination among treatments.

Five different vinification experiments were conducted. Thus, grapes from plots B and C (i.e., those treated with boscalid + kresoxim-methyl and metrafenone, respectively) were subjected to direct vinification in triplicate. On the other hand, grapes from plot A were split into three batches (A, D and E). Batch A was vinified directly ($n = 4$) and used as control, whereas batches D and E were spiked in an experimental cellar ($n = 3$) with the commercial products Frupica® (50% w/w mepanipyrim, Sipcam Iberia, Valencia, Spain), and Teldor (50% w/w fenhexamid, Bayer CropScience, Valencia, Spain), respectively, prior to vinification. The spiking concentrations used were their respective MRLs in vinification grapes as established in Commission Regulation (EC) No. 149/2008, namely: 3 mg kg^{-1} for mepanipyrim —the current the MRL for this pesticide as set in Commission Regulation (EU) No. 777/2013 is 2 mg kg^{-1}, however— and 5 mg kg^{-1} for fenhexamid.

The winemaking processes were conducted under identical conditions in the experimental cellar of the University of La Rioja (Spain) (Briz-Cid et al., 2015). Thus, crushed, destemmed grapes were placed in metallic fermentation vessels and supplied with sulphite (50 mg SO_2 L^{-1}). The temperature was kept at 17–21 °C throughout alcoholic fermentation. After 14 days, the wine was transferred to other vessels and grape residues were pressed. The SO_2 content of the wine was then adjusted by adding 30 mg SO_2 L^{-1} to each vessel. All wines were subjected to cold clarification prior to bottling. No significant differences at $p < 0.05$ in the evolution of alcoholic fermentation (sugar contents) among treatments were observed. In fact, only the wines made from grapes treated with boscalid + kresoxim-methyl exhibited a slight initial delay —the process, however, finished concurrently with the others. A proportion of 82–93% of the initial amounts of fungicide residues dissipated during the winemaking process (Noguerol-Pato et al., 2016).

2.2. Characterization of colour fraction, anthocyanin distribution and phenolic fraction

Wine colour was determined from CIELab parameters (OIV, 2000). Co-pigmented, monomeric, polymeric and total anthocyanins were determined according to Boulton (1996). The procedures used to extract anthocyanins, flavan-3-ols, phenolic acids and flavonols are described in detail elsewhere (Briz-Cid et al., 2014). The CIELab space includes one channel for lightness (L^*) and two for colour (a and b). The a-axis spans from green (−a) to red (+a) and the b-axis from blue (−b) to yellow (+b). These colour channels were used to obtain the cylindrical coordinates C_{ab}^* (chroma, relative saturation) and h_{ab} (hue angle). The total content in anthocyanins (A^{acet}) and their distribution into the monomeric (A^{20}–A^{SO2}), polymeric (A^{SO2}) and co-pigmented fractions ($A^{acet} - A^{20}$) were determined according to Boulton (1996). This entailed adjusting the pH of the wine to 3.6 and measuring the absorbance at 520 nm of the following mixtures: A^{acet} [2 mL of wine + 20 µL of 10% (v/v) acetaldehyde], A^{20} [100 µL of wine + 1.9 mL of 12% (v/v) ethanol] and A^{SO2} [2 mL of wine + 160 µL of 5% (w/v) SO_2].

2.2.1. Anthocyanins

Once all ethanol was evaporated, 2 mL of wine reconstituted with water was loaded onto a Strata C18 cartridge that was previously activated with methanol (10 mL) and water (10 mL). The cartridge was then dried with N_2 for 30 min and washed with ethyl acetate (20 mL) prior to eluting the anthocyanin fraction with 0.1% TFA in methanol (30 mL). The eluate was evaporated to dryness and redissolved in 12% (v/v) ethanol, the resulting extract being passed through a filter of 0.45 µm pore size for analysis by HPLC/DAD–ESI/MS according to Briz-Cid et al. (2014). A Thermo Separation-Products (TSP, Waltham, MA, USA) system equipped with a TSP SCM1000 vacuum membrane degasser, a P2000 binary pump, a TSP AS1000 autosampler and a UV6000LP diode array detector operating from 200 to 600 nm were also used. The chromatographic column was a Phenomenex C18 Luna analytical model (150 × 3 mm, 5 µm i.d.) furnished with a Pelliguard LC-18 guard column (50 × 4.6 mm, 40 µm i.d.) from Supelco (Bellefonte, PA, USA). The chromatographic system was coupled to a TSQ Quantum Discovery triple-stage quadrupole mass spectrometer from Thermo Fisher Scientific (Waltham, MA, USA) for confirmatory analyses. The spectrometer was operated in the negative electrospray ionization (ESI) mode under the following conditions: spray voltage 4000 V; capillary temperature 250 °C; sheath gas and auxiliary gas pressure 30 and 10 units, respectively; collision energy 25 and tube lens offset 110. Samples?? were acquired over the m/z range 100–1700 in the full-scan mode, and also in the MS/MS mode.

2.2.2. Phenolic acids and flavonols

After all ethanol was evaporated, 3 mL of reconstituted wine was adjusted to pH 7 and loaded onto an MCX cartridge that was previously activated with methanol (5 mL) and water (5 mL). The cartridge was washed with 0.1 M hydrochloric acid (5 mL) and water (5 mL), and then used to elute the analytes with methanol (15 mL). The eluate was evaporated to dryness and redissolved in 12% (v/v) ethanol. Then, the extract was passed through a filter of 0.45 µm pore size for analysis by