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Phenolic compounds and antioxidant activity of red wine made from grapes treated with different fungicides

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ABSTRACT

The effect of treating grapes with six fungicides, applied under critical agricultural practices (CAP) on levels of phenolic compounds and antioxidant activity of red wines of Monastrell variety was studied. Vinifications were performed through addition of active dry yeast (ADY). Measurement of phenolic compounds was made with HPLC-DAD. Determination of antioxidant activity was through reaction of the wine sample with the DPPH· radical. The wine prepared from grapes treated with quinoxyfen shows a greater increase of phenolic compounds than the control wine. In contrast, the wine obtained from grapes treated with trifloxystrobin showed lower total concentration of phenolic compounds, including stilbenes, whilst treatments with kresoxim-methyl, fluquinconazole, and famoxadone slightly reduced their content. Hence, the use of these last four fungicides could cause a decrease in possible health benefits to consumers. Antioxidant activity hardly varied in the assays with quinoxyfen, fluquinconazole and famoxadone, and decreased in the other wines.

1. Introduction

Phenolic compounds appear as the grape changes colour, substituting the chlorophyll. They are of great oenological importance and play a key role in determining the quality of the wine. Along with their nutritional and pharmacological properties they also account for characteristics like colour, aroma, taste and astringency (Bartolomé, Núñez, Monagas, & Gomez-Cordovés, 2004; Harborne & Baxter, 1999). Their antioxidant properties also have positive effects on a wine's stability (Cheynier, 2001; Waterhouse, 2002). The total content of polyphenols is also an indication as to whether the wine can be aged.

Grapes of the *Vitis* type are relatively rich in phenolic compounds compared to other edible fruits. The grape essentially contains non flavonoid compounds in the pulp and flavonoid compounds in the skin, seeds and stems. It is estimated that seeds contain 65% of the polyphenols of the bunch, the stem 22%, the skin 12% and the pulp just 1% (Hidalgo Togores, 2003). Hence, the technological transformation the grape undergoes conditions the extraction of these compounds and, therefore, contributes to the polyphenolic composition of the wines.

Many factors affect the concentration of phenolic compounds in the grape and the wine: the growth state, the variety, environmental conditions, diseases and treatments of the vines, both with fungicides and with elicitor SAR compounds and plant resistance inducers, as well as edaphoclimatic features and ripeness (Gil & Yuste, 2004; Oliva, Barba, San Nicolás, & Payá, 2005; Oliva, Navarro, Salinas, Barba, & Navarro, 1999; Ruiz-García et al., 2012; San Nicolás, Oliva, Barba, Fernández, & Salinas, 2002; Tregoat, Van Leevwen, Chone, & Gaudillere, 2002; Vitalini et al., 2011). Other processes affecting concentrations include the time and temperature of the maceration of the wine, the presence of SO_2, pH, copigmentation and micro-oxygenation phenomena, etc. (Gao, Girard, Mazza, & Reynolds, 1997; Gómez-Plaza, Gil-Muñoz, López-Roca, & Martínez, 2000).

Vinification involves musts and wines being in constant evolution. The phenolic composition of the wine depends on the raw material and the type of vinification followed, which affects physical phenomena (diffusion from the solid parts, extraction of wood compounds, etc.), and chemical and biochemical phenomena (oxidation, degradation, condensation, etc.). Throughout vinification there are exogenous enzymatic activities of various natures which may come from micro-organisms present in the vinification or from oenological enzymatic preparations. In both cases they may be affected by the presence of pesticides, in particular fungicides, used during growing, (Oliva et al., 2005).

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The phenolic compounds are an integral part of human diet and are considered to be non nutrient biologically active compounds (Subramani, Casimir, & Krewer, 2002). Being polyphenolic compounds, flavonoids are able to act as antioxidants through a mechanism that removes free radicals and by chelation of metal ions (Sellappan, Akoh, & Krewer, 2002). The growing evidence for the role of radicals and antioxidants in health and ageing and the importance of wine in the Mediterranean diet has aroused much interest in these compounds. A wide range of studies has shown that the antioxidant properties of these compounds can offset atherosclerosis and coronary heart disease whilst showing selective cytotoxicity to breast cancer cells (Obrenovich et al., 2011; Walter et al., 2008; Xiang et al., 2014).

Research into wine growing is essential in advancing in this complex scientific field of the behaviour of phenolic substances and so optimise growth practices, amongst them the application of plant protections. One of the least studied of these factors is without doubt the possible effect of pesticide residues present in grapes and wine on phenolic composition.

Nowadays, several fungicides are used to control diseases in grapes: famoxadone, oxazolidinone known for its effective, wide-ranging prevention of various foliar fungal diseases (Bartlett et al., 2002; Jernberg, 2003); fenhexamid, hydroxyanilide used in the control of *Botrytis cinerea* in grapes (Duben, Rosslenbroich, & Jenner, 2002); fluquinconazole, thiazole used in the control of *Uncinula necator* (Metcalfe, Shaw, & Russell, 2000); kresoxim-methyl, oxymino acetate (strobilurin) used to control *U. necator* in grapes (Grossmann & Retzlaff, 1999); quinoxyfen, a quinoline used in grapes to control powdery mildew (*U. necator*) (Wheeler et al., 2003); and trifloxystrobin, an active strobilurin to combat powdery mildew in grapes (De Melo, Correia, Herbet, Santos, & Alves, 2005).

Given the above, this paper seeks to relate the effect of these six currently used fungicides under CPA on the content of phenolic compounds and the antioxidant activity in red wines of the Monastrell variety belonging to the Jumilla Appellation d'Origine Contrôlée (Jumilla, Murcia, SE Spain).

2. Materials and methods

2.1. Fungicides

The following active substances were used: fenhexamid (2,3-dichloro-4-hidroxy-1-methylcyclohexanecarboxanilide), kresoxim-methyl [methyl (E)-methoxyimino[2-(o-tolyloxymethyl) phenyl] acetate], fluquinconazole [3-(2,4-dichlorophenyl)-6-fluoro-2-(1H-1,2,4-triazol-1-yl)quinazolin-4(3H)-one], famoxadone [3-anilino-5-methyl-5-(4-phenoxyphenyl)-1,3-oxazolidine-2,4-dione], trifloxystrobin [methyl(E)-methoxyimino-{(E)-α-[1-(α,α,α-trifluoro-m-tolyl)ethyl ideneaaminooxy]-0-tolyl}acetate] and quinoxyfen (5,7-dichloro-4-quinolyl-4-fluorophenyl ether); purchased as analytic standards from Dr. Ehrenstorfer (Germany) and commercially prepared by Bayer Hispania S. A., Basf Española S. A., Du Pont de Nemours & Co, Bayer CropScience S. L and Dow AgroSciences Ibérica.

Trade names and main features are given in Table 1.

2.2. Chemicals and reagents

The following products were used: formic acid, methanol and ethyl acetate for chromatography (Merck, Darmstadt, Germany), cyanidin 3-rutinoside (Polifenoles AS, Sandnes, Norway), rutin (Merck, Darmstadt, Germany), chlorogenic acid (Sigma, Madrid, Spain), trans-resveratrol (Sigma, Madrid, Spain), Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and 2,2-diphenyl-1-picrylhydrazyl (DPPH·) (Sigma, Steinheim, Germany). Ultrapure water obtained using a Milli-Q System (Millipore Corp., Bedford, MA).

2.3. Equipment

Spectrophotometer UV–VIS 190–1100 nm, Varian Cary 50, carrying Software WinUV (Victoria, Australia). 0.45 μm filters, Millex HV13 type (Millipore Corp, Bedford, MA). High performance liquid chromatography with diode-array detection (HPLC-DAD), coupled with a Merck-Hitachi L-6200 pump (Merck-Hitachi, Darmstadt, Germany) and diode-array detector Shimadzu SPD-M6A UV (Shimadzu, Kyoto, Japan) equipped with an reverse phase column, Lichrospher® RP 100-18 (25 × 0.4 cm, 5 μm particle size) (Merck, Darmstadt, Germany).

2.4. Plant materials

The study was carried out at a red grape plantation (*Vitis vinifera*, var. Monastrell) aged 25–30 years, in a plot of 2.5 × 2.5 m, located in Jumilla, Murcia (SE Spain). Plants were in perfect nutritional state and showed excellent physiological conditions. Seven experimental plots of 225 m^2 were chosen (36 Monastrell vine stocks per plot) labelled P1–P7. One (P1) was chosen as the control plot, whilst plots 2–7 each received treatment of one fungicide under CPA, on the day of grape collection.

Grapes were harvested in October with 50 kg of grape harvested from each plot following FAO (FAO, 1990) sampling recommendations. The grapes were then transported to the experimental winery, where the 50 kg of grape from each plot were divided into three sub-samples of 15 kg, in order to make three vinifications of each plot.

2.5. Wine-making

Using the seven samples of harvested grapes, seven micro-vinifications were made in triplicate with 15 kg of grape from each plot. All the vinifications were made with the addition of ADY for the purposes of maximum homogenisation.

Vinification was performed at the pilot date on the same day as harvesting. The bunches of grapes were passed through a compression roller. After the removal of the stems, the crushed grapes were

Table 1
Pesticide treatments, dose, active ingredient by Ha and pre harvest intervals (PHI).

Fungicide	Commercial name	% a.i.	Appl. dose (%)	a.i./Ha (g)	PHI (days)
Fenhexamid	Teldor 50 WG	50	0.1	450	14
Kresoxim-methyl	Stroby WG	50	0.02	45	35
Fluquinconazole	Castelan GD	25	0.025	28	21
Famoxadone	Equation Pro GR	22.5	0.04	45	28
Trifloxystrobin	Flint 50 WG	50	0.015	34	28
Quinoxyfen	Arius SC	25	0.03	34	28

%a.i. = % active ingredient; Appl. Dose = % applied dose; a.i./Ha = g active ingredient/Ha; PHI = days pre-harvest interval.