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Research Note

Pre-harvest spraying with rutin improves colour of 'Tempranillo' grapes and wines

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Introduction: The coloured forms of anthocyanins can be stabilized by other natural components, the so-called copigments, which exist in the cells of flowers and fruits (Brouillard 1982). The formation of copigmentation complexes between anthocyanins and copigments, usually non-coloured phenolics, is one of the major factors affecting the colour of young red wines (Boulton 2001), inducing a hyperchromic (higher absorbance values) and bathochromic effect (higher wavelength at which the maximum absorbance is observed in the absorption spectra). The copigmentation phenomenon depends on the nature of anthocyanins and copigments, the copigment-anthocyanin ratio and the pH of wine (Mazza and Brouillard 1990).

Some authors have shown enhanced colour intensity and stability in young red wines made with prefermentative addition of cofactors as rutin, catechin and caffeic acid (Álvarez *et al*. 2009, Schwarz *et al*. 2005). Rutin is a flavonol, the group of polyphenols considered to be one of the best copigments (Boulton 2001). The application of copigments in vineyard, directly on clusters, could promote the copigmentation in grapes to improve chromatic features of wines. In this respect, sprayings of natural extracts from plants rich in copigments (e.g. buckwheat flour in the case of rutin) could be very interesting for organic viticulture.

Alonso *et al*. (2007) showed positive effects of caffeic acid treatments in the field on 'Tempranillo' grape colour when the product was applied 15 d before harvest. Neither effects of field sprayings with other copigments nor effects of treatments on wine colour stability have been reported yet. The aim of this work was to determine the influence of rutin, applied over clusters in different doses two weeks before harvest, on the polyphenol content and chromatic characteristics of 'Tempranillo' grapes and wines.

K e y w o r d s : anthocyanins, copigmentation, grape composition.

Material and Methods: The study was carried out in 2004 and 2005, in a commercial 'Tempranillo'/110 Richter vineyard located in 'Ribera del Duero' *Appellation d'Origine* area (Northern Spain). The vines (2200 per ha) were trained to a double cordon Royat system with four spurs of two buds on each arm. Rainfall recorded during 2004 and 2005 was 454 and 341 mm respectively, and the mean temperature during ripening was close to 19 ºC in both years. Due to drought conditions, the average yield was only 2.6 kg·vine^{-1}.

The trial was designed in a randomized complete block with four replications. Base plots (with five vines in each of them) were treated with a solution of 0, 500 or 1000 mg·L^{-1} rutin (Sigma-Aldrich, ≥ 95 % HPLC) dissolved in an hydro-alcoholic solution of ethanol 15 % v/v. The treatments were applied on the clusters 15 d before harvest using a hand sprayer, 100 berries from each experimental treatment were randomly collected just before harvest, removing the skins from pulps and seeds. The must composition parameters were analyzed according to the official methods in the European Union. The solid fraction of the samples was subjected to a process of extraction of polyphenolic compounds as described in Delgado *et al.* (2006), to finally determine the total polyphenol and anthocyanin contents.

In 2005, wines from experimental treatments were made as follows. The trial was harvested when average potential ethanol content was 12.9 % and total acidity 6.72 g·L^{-1} (as tartaric acid).The grapes were destalked and crushed, and 100 mg of SO_2 (as $K_2S_2O_5$)·kg^{-1} was added to resulting mash to avoid oxidation. After soft pressing, the mash (5 kg per treatment) was transferred to glass jars. Here, alcoholic fermentation was made at 22 ºC controlled temperature by inoculating commercial yeast. Manual punching down (pigeage) was carried out twice a day. The malolactic fermentation was induced by inoculation with *Oenococcus oeni*. Finally, after a natural decanting, the wines were bottled and stored in a condition room kept at 13.5 °C until analysis.

CIELAB ccoordinates of skin extracts and wines were determined from the spectra acquired with a UV/VIS JascoV-530 spectrophotometer. The contribution of total, copigmented, not copigmented and polymerized anthocyanins to wine colour were quantified following Boulton (2001) methods. All wine samples were analyzed after bottling, and after 3, 6 and 9 months of storage. The effects of experimental treatments on grape and wine parameters were studied by analysis of variance and Least Significant Difference (LSD) test.

Results and Discussion: The application of rutin over clusters did not modify total soluble solids content (mean values between 22.8 and 23 ºBrix) and pH (from 3.53 to 3.67) of musts at harvest. However, rutin adhered on surface and/or translocated into grape after treatments significantly increased total polyphenols and total anthocyanin contents in skins (Table). The sprayings enhanced chroma in skin extracts with respect to controls (hyperchromic effect) independently of dose, and increased blue component of colour (bathochromic effect), as reflects b* coordinate in Table. Both effects could be produced by an increase in the proportion of copigmented anthocyanins, which could provide a greater protection of the anthocyanins against oxidation in wines (Boulton 2001).

With larger signification levels than for skin extracts, wines from treated grapes in 2005 after bottling also ex-

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T a b l e

Total polyphenol and anthocyanin contents in grapes, and CIELAB coordinates of skin extracts according to the rutin treatments applied

Year	Rutin dose ($mg \cdot L^{-1}$)	Total Polyphenols ($mg \cdot kg^{-1}$)	Total anthocyanins ($mg \cdot kg^{-1}$)	a*	b*	C*	L*
2004	0	828 a	1208 a	25.8 a	0.03 a	30.9 a	71.8 a
	500	844 a	1537 a	28.6 a	-0.48 b	34.4 b	73.2 a
	1000	1044 b	1709 b	20.9 a	-0.57 b	35.7 b	79.8 a
2005	0	739 a	1030 a	32.2 a	-0.09 a	20.37 a	67.2 b
	500	939 b	1208 b	26.8 a	-1.36 b	26.98 b	74.9 a
	1000	960 b	1278 b	24.2 a	-1.91 b	28.07 b	73.2 ab

Values followed by the same letter are not significantly different ($p < 0.05$).

hibited higher total anthocyanin content ($693\ mg \cdot L^{-1}$) and colour intensity (19.7) than controls ($663\ mg \cdot L^{-1}$ and 19.5 respectively). Similar results have been shown for Tempranillo wines obtained after prefermentative (Alvarez *et al.* 2009) and postfernentative (Schwarz *et al* 2005) addition of rutin. Probably, the increases in total anthocyanin contents for skin extracts and wines we detected were due to a greater proportion of anthocyanins in copigmented forms, these being more stable during polyphenol extraction and winemaking, and having remained in the medium without precipitating (Schwarz *et al*. 2005).

After nine months of storage, the wines lost up to 48 % of their colour intensity at bottling (Figure). The percentage of wine colour due to copigmented anthocyanins also decreased along the storage period, but samples from treated grapes showed significantly higher values than controls,

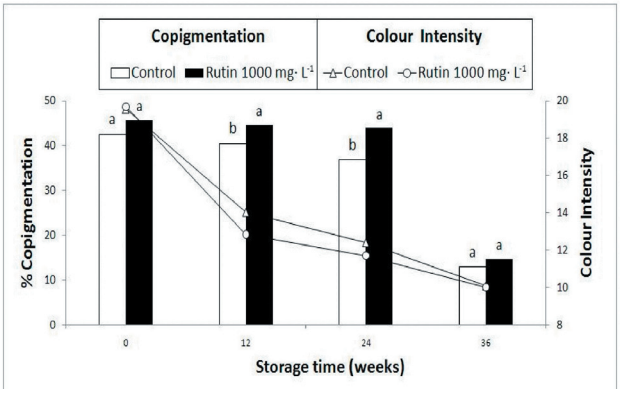

Figure: Colour intensity and copigmentation percentage during the storage period in wines coming from control grapes and treat-

at least until six months from bottling (in this point differences in colour intensity are maximum). On the other hand, rutin treatments increased the percentage of anthocyanins inside of polymeric pigments in wines three months after botling (average values of 14.2 % for 1000 $mg \cdot L^{-1}$ dose vs 11.72 % in untreated controls), as reported by Alonso *et al.* (2007) for caffeic acid applications in the field. The promoting of copigmentation phenomena is very interesting in young red wines because they contribute to more colour intensity and blue components of colour for longer time. Moreover, the promoting of anthocyanin polymerization reactions detected could improve the colour stability of wine in future aging processes.

Our results have shown that rutin spraying over the clusters at the end of ripening, can increase polyphenol and anthocyanin contents in grapes and wines, contributing to improve colour intensity and stability. The treatments in vineyard could be, at least, so interesting as the prefermentative addition to must reported previously.

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