

Technical Report

Effect of p-Coumaric Acid on the Color in Red Wine

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Caffeic and p-coumaric acid were added as copigments to 'Cabernet Sauvignon' and 'Pinot noir' grapes after crushing. The respective wines were analyzed on the influence of these copigments on color by means of spectrophotometer. Generally, p-coumaric acid was more effective in producing a hyperchromic shift towards higher absorptions at 520nm and an hypochromic shift towards lower wavelengths. It is thus concluded that optimal delocalisation of the π -electrons of the copigment seems to be partially responsible for the stronger copigmentation effect of p-coumaric acid compared to caffeic acid.

Keywords: wine; copigmentation; anthocyanin; p-coumaric acid; caffeic acid; polyphenols; color

The color of red wine depends on a number of different parameters. One important parameter is the so-called copigmentation effect. This phenomenon involves the loose association between anthocyanins and other (mainly colorless) compounds, so-called copigments, or even a covalent bonding between both (BLOOR and FALSHAW, 2000; DANGLES et al., 1993; BROUILLARD, 1981). The colorless copigments associate with the colored anthocyanin flavylium-ion, shifting the anthocyanin equilibrium to the flavylium-ion side, which leads to increased color (hyperchromic effect) (BARANAC et al., 1996; MIRABEL et al., 2001). Recent research has shown that copigmentation can account for 30 to 50 % of the color in red wine and is thought to be the first step in the formation of new pigments in aged wine (BOULTON, 2001; MATEUS and DE FREITAS, 2001; MATEUS et al., 2001).

In the past, research mainly focussed on tannins and flavan-3-ols and their effect on copigmentation and the formation of new pigments in wine (BARANAC et al., 1996; MIRABEL et al., 2001). But recent research has suggested that hydroxycinnamic acids play a much more important role in copigmentation than tannins. Caffeic

acid has been shown to have a strong copigmentation effect on red wine (DARIAS-MARTIN et al., 2001; DARIAS-MARTIN et al., 2002) and on cyanidin-3,5-glucoside in model solutions (ASEN et al., 1972). In regard to the general hydroxycinnamic acid composition of red wine, p-coumaric acid is often found in higher concentrations than caffeic acid (PENA-NEIRA et al., 2000). To our knowledge no data exist on the effects of p-coumaric acid on color development in red wine.

Materials and methods

Wine

'Cabernet Sauvignon' and 'Pinot noir' grapes were obtained from a commercial vineyard in the Canterbury region in the South Island of New Zealand. After crushing the grapes received 60 mg/l of sulfur dioxide and then were divided into three groups and further into three batches of 2 liters each. In the first group each batch received 150 mg/l p-coumaric acid and in the second group each batch received 150 mg/l caffeic acid.

The final group, also consisting of three batches, was kept untreated as a control.

The batches were all inoculated with Enoferm T306 (Lallemand Inc., Montreal, Canada) and fermented at 22°C. Plunging was performed three times a day. After fermentation was completed lactic acid bacteria starter culture (*Viniflora oenos*, CHR Hansen, Hornsholm, Denmark) was added to induce malolactic fermentation. The wines were pressed after seven days, when the primary fermentation was completed. After malolactic fermentation was concluded 70 mg sulfur dioxide per liter were added and the wines were stored in the dark at 12 °C until further analyses. Samples were analyzed at three points of time: after malolactic fermentation was complete (40 days after addition of copigment), and after 60 and 90 days after addition of copigment.

VIS Absorption Spectroscopy

The absorbance was measured between 380 and 700 nm with a bandwidth of 2 nm. Spectra were recorded on a Unicam (UV4) UV/VIS spectrometer (Vision software) in Eppendorf UVette 2 mm-cuvettes.

Results and discussion

'Pinot noir'

Immediately after completion of malolactic fermentation caffeic acid showed the strongest hyperchromic

Table 1:

Mean values (n=3) of color enhancement in 'Pinot noir' after addition of hydroxycinnamic acids

Days after addition	Control		Caffeic acid		p-Coumaric acid	
	absorbance (520nm)	λ_{max} (nm)	absorbance (520nm)	λ_{max} (nm)	absorbance (520nm)	λ_{max} (nm)
40	0.2790 ± 0.021	526	0.4700 ± 0.161 (+68.5%)	524	0.4333 ± 0.038 (+55.3%)	514
60	0.3337 ± 0.033	526	0.4627 ± 0.044 (+38.7%)	528	0.4923 ± 0.039 (+47.5%)	514
90	0.4553 ± 0.060	526	0.5433 ± 0.040 (+19.4%)	526	0.6360 ± 0.025 (+39.7%)	518

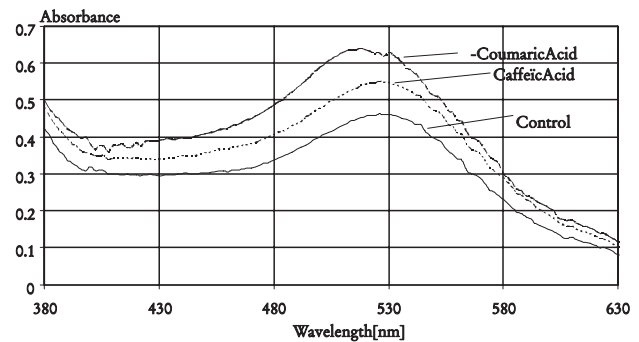


Fig. 1: Influence of copigments on color in 'Pinot noir' (90 days after addition of copigment)

shift in 'Pinot noir' followed by p-coumaric acid, the latter tending towards lower wavelengths with a maximum at 514 nm compared to 526 nm for the control (i.e., hypsochromic shift, Table 1). The hyperchromic shift evoked by caffeic acid was quite significant. There was a 68.5 % increase in absorption at 520 nm after 40 days compared to the control, while for p-coumaric acid the increase was 55.3 %. After 60 days the impact of caffeic acid on copigmentation was weaker than for p-coumaric acid but overall there was an increase of 38.7 % and 47.5 %, respectively, compared to the control. After 90 days another increase was observed, which again was larger for p-coumaric acid (39.7 %) than for caffeic acid (Fig. 1). Compared to the control caffeic acid had only an about 19.3 % stronger absorption at 520 nm.

'Cabernet Sauvignon'

Directly after malolactic fermentation a strong increase in color was observed in the p-coumaric acid treated 'Cabernet Sauvignon' wine (29.6 %), while caffeic acid had a slightly lower absorbance at 520 nm than the control (-4.1 %), (Table 2). No hypso- or bathochromic shift was observed for either treatment. After 60

Table 2:
Mean values (n=3) of color enhancement in 'Cabernet Sauvignon' after addition of hydroxycinnamic acids

Days after addition	Control		Caffeic acid		p-Coumaric acid	
	absorbance (520nm)	λ_{\max} (nm)	absorbance (520nm)	λ_{\max} (nm)	absorbance (520nm)	λ_{\max} (nm)
40	0.4533 ± 0.029	526	0.4347 ± 0.116 (-4.1%)	526	0.5877 ± 0.029 (+29.6%)	526
60	0.6533 ± 0.066	528	0.7147 ± 0.038 (+9.4%)	526	0.7623 ± 0.046 (+16.7%)	526
90	0.7060 ± 0.048	528	0.7373 ± 0.053 (+4.4%)	526	0.7947 ± 0.046 (+12.6%)	526

days there was a much smaller impact from copigmentation seen on the color compared with immediately after malolactic fermentation. The increase in color compared to the control was again strongest for p-coumaric acid (16.7 %) followed by caffeic acid (9.4 %). Again no hypso- or bathochromic shift was observed. After 90 days p-coumaric acid showed a 12.6 % increase compared to the control, while caffeic acid only showed a 4.4 % increase in color at 520 nm.

These data suggest that the positively charged flavylum-ion could be better stabilized by the optimally delocalised π -electrons of p-coumaric acid rather than caffeic acid. These results, therefore, are in opposition to the data published by ASEN et al. (1972), who found caffeic acid to have a much stronger bathochromic effect compared to p-coumaric acid. The latter also showed a weaker hyperchromic effect than caffeic acid, while in our studies both compounds showed a hypsochromic shift and p-coumaric acid was generally more effective. Furthermore, our results support the data recently published by EIRO and HEINONEN (2002).

In contrast to 'Pinot noir', which contains no acylated anthocyanins, acylation of the sugar moiety in 'Cabernet Sauvignon' could - partially - explain the smaller color stabilizing effect in the latter variety. Steric hindering could occur through a „folding“ of the acylating molecule (e.g., hydroxycinnamic acids) leading to fewer opportunities for copigments to place themselves in a position horizontal to the anthocyanin molecule and, thus, not allowing complete overlapping of the π -bonds, which would stabilize the positive charge on

the anthocyanin molecule. Furthermore, chemically and/or enzymatically formed vinylphenols from p-coumaric acid could also react with the anthocyanins thereby stabilizing their color. Therefore, research should focus on this compound and its ability to stabilize color in color sensitive cultivars from cool-climate vine-growing areas such as 'Pinot noir' from New Zealand.

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