Effect of Caffeic Acid on the Color of Red Wine

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The copigmentation effect of prefermentation additions of different doses of caffeic acid was
investigated during the 1997 harvest. Microfermentation with the major red grape cultivars Listán
negro and Negramoll, grown in the Canary Islands, was carried out with the same protocol. Visible
and UV spectra were registered periodically. HPLC chromatograms were carried out. The color
enhancement of cv. Negramoll wine varied between 13 and 75% (AU at 520 nm), and that of cv.
Listán negro wine between 25 and 45% at the end of fermentation. During aging these values were
enhanced to reach even >100% in some cases. An initial complex of the 1:1 type, where one molecule
of caffeic acid associates with one molecule of anthocyanin, has been identified using the mathematical
procedure of Brouillard et al. (*J. Am. Chem. Soc.* **1989**, *111*, 2604−2610). Caffeic acid seems to
contribute to color stability and protection against oxidation. The importance of nonpigment composition
in pigment extraction and color retention during and after fermentation is demonstrated.

KEYWORDS: Copigmentation; caffeic acid; red wine; UV−visible spectrophotometer; color wine

INTRODUCTION

The color of a red wine is a result of the constituents of the
grape used, the wine-making techniques, and the stabilization
and aging procedures employed. Anthocyanins are to a large
degree responsible for the color of young red wines, playing a
fundamental role despite the fact their reactivity causes them
to fade away during aging. Reactions that involve the oxidation
of the chalcone forms of anthocyanins with irreversible color
loss have been proposed (*1*, *2*). The association between
anthocyanins and flavanols is known as "polymeric pigments"
(*3*, *4*), which stabilize wine color. More recently interest has
been focused on the copigmentation reactions between antho-
cyanins and diverse cofactors or copigments (*5*−*9*). In an
attempt to understand the transformations occurring in wine
color, a series of studies were carried out in model solutions as
a preliminary approach to this topic. Thus, copigmentation,
anthocyanin−flavanol condensation, and flavanol browning
reactions have all been studied in aqueous and aqueous−
alcoholic media (*9*−*28*). In other studies, the importance of
condensation with acetaldehyde as a bridge between anthocya-
nins and flavan-3-ols has been highlighted (*28*−*34*). However,

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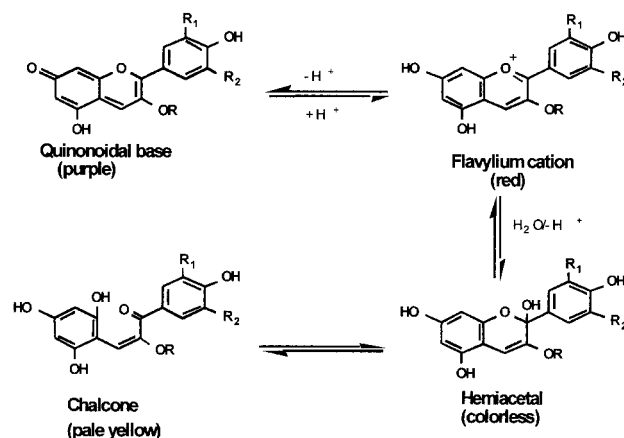versidad de La Laguna.
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Figure 1. Equilibrium of anthocyanins in acidic aqueous solutions.

these processes are not so easy to demonstrate in a more
complex medium such as wine. For this reason, some of the
hypotheses put forward to explain the changes occurring during
red wine aging have been speculative.

In water/alcohol solutions, as in wine, anthocyanins exist as
different forms in equilibrium, conditioned by their pH (**Figure
1**). Of these, only the flavylium cations that predominate at very
acid pH are red in color (*1*, *35*); therefore, at the pH of wine
(3.2−4.0) most of the anthocyanins are in their colorless or
faintly colored forms. One process that may be of great
importance that counteracts the negative effect of the relatively

Effect of Caffeic Acid on Red Wine Color

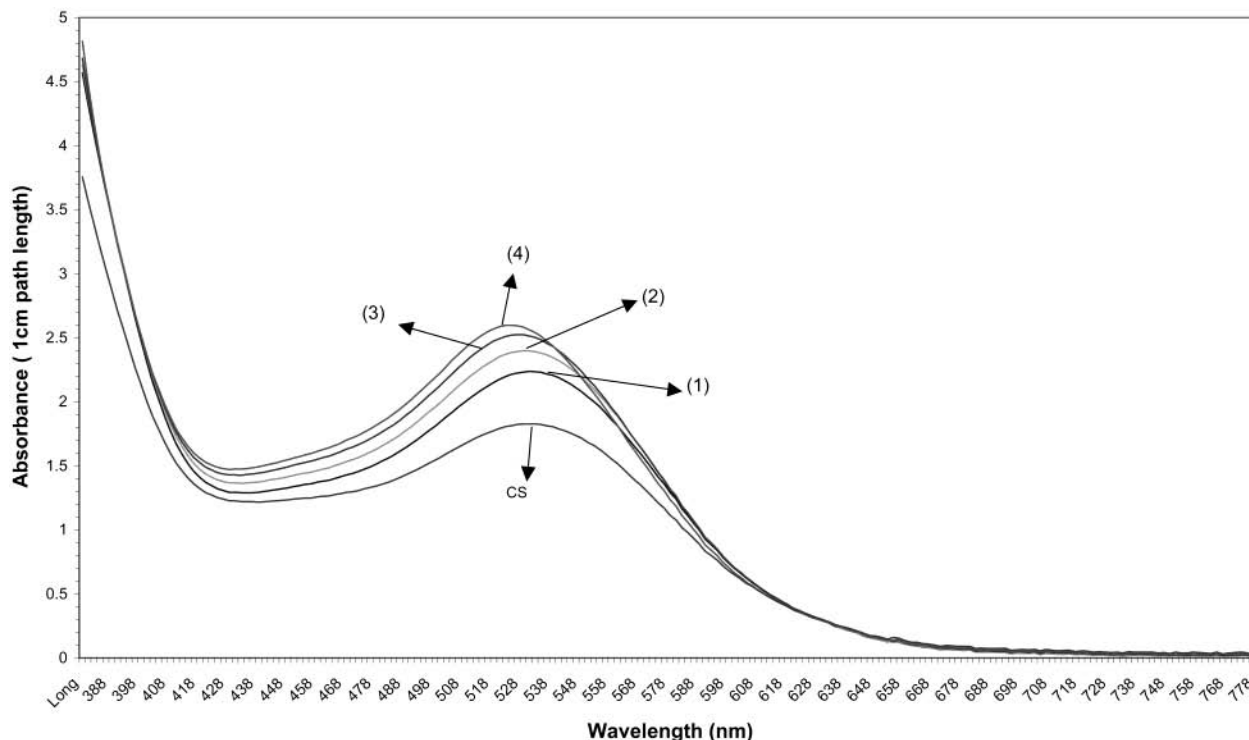*J. Agric. Food Chem.,* Vol. 50, No. 7, 2002 **2063**

Figure 2. Visible spectra of cv. Listán negro wines after alcoholic fermentation: CS, control; (1) 120 mg/L; (2) 240 mg/L; (3) 480 mg/L; (4) 960 mg/L.

low acidity of wine is copigmentation, which contributes to the extraction and conservation of the red color in young red wines where anthocyanins are still largely responsible for color, serving perhaps as a first step in the formation of more time-stable pigments during wine aging (*7, 24*). As already mentioned, these copigmentation phenomena have been widely studied in model solutions to determine the influence of factors such as pH, temperature, alcohol content, and copigment/pigment molar ratios on the magnitude and extent of copigmentation. Thus, an increase in temperature or alcohol content diminishes the effect of copigmentation and the copigment/pigment molar ratio increases. The copigment, normally a colorless organic compound, associates predominantly with the colored forms of anthocyanins, displacing their equilibria toward colored forms of the flavylium cation. This increases color intensity (hyperchromic effect), producing a displacement of the wavelength at which maximum absorbance occurs (bathochromic effect). This is easily confirmed by absorption spectrophotometry in the visible range.

The copigment complex is stabilized by the surrounding sugar molecules joined by external hydrogen bridges, which protects it from hydration and therefore from displacement toward the colorless form of the carbinol base (*36*).

To elucidate the role of this type of reaction in wine production, a preliminary trial was carried out (*8*), demonstrating the effectiveness of caffeic acid in the enhancement of red wine color. In the present study, we approach its effect on two red cultivars by adding different prefermentation doses of this copigment to the must. In this way we progress from model solutions to real wine-making in order to confirm the extent of copigmentation phenomena in red wines. A periodic followup was performed by UV−visible spectrophotometry. HPLC chromatograms were carried out to confirm the formation of new pigments. To our knowledge, there are only a few studies in the literature concerning copigmentation in red wine production.

MATERIALS AND METHODS

Wine-Making Procedures. The wines were made in the experimental wine production center at La Laguna University. The red cultivars Listán negro and Negramoll used are those most widespread in the Canary Islands. For each of them the following procedure was carried out: the grapes were harvested manually with ∼20 °Brix of ripeness, crushed and destemmed, then placed in their entirety in a 1000 L stainless steel tank, where they were homogenized, with the addition of 40 mg/L potassium metabisulfite. The must was then distributed into 50 L stainless steel containers, where it was inoculated with selected commercial *Saccharomyces* yeast. Caffeic acid was added inmediately at doses of 120, 240, 480, and 960 mg/L. The batches were run in triplicate, keeping control samples of each variety. The length of extraction was 72 h, maintaining the temperature at 25 °C and measuring density daily. When the alcoholic and malolactic fermentations were completed, the wines were racked, with sulfur dioxide (60 mg/L) added as potassium metabisulfite. During winemaking no clearing agents or pectolytic enzymes were used. The wine was racked periodically and bottled three months after its preparation.

UV−Visible Absorption Spectroscopy. Visible absorption spectra (380−780 nm) and UV absorption spectra (200−380 nm) of wines with and without caffeic acid were recorded periodically on a Perkin-Elmer Lambda 11 spectrophotometer. Quartz cells of between 1 mm and 1 cm path length were used. The mathematical procedure of Brouillard et al. (*17*) was used to calculate the color enhancement and numbers of molecules in the complex.

HPLC Analyses. Before analysis, the samples were filtered through a Waters 4 mm PTFE 0.45 μm filter.

A Hewlett-Packard (HP) 1050 gradient liquid chromatograph with a DAD 1050 M coupled to a Chemstation HP Rev Asterix 02.05 was used. The column, a Nucleosil 120 C_{18} (250 mm × 4 mm), 5 μm particle size, was kept at 40 °C. The volume injected was 100 μL, by means of an HP 1050 automatic injector.

A constant flow rate of 1.5 mL/min was used with two solvents: solvent A, 2.7% glacial acetic acid in water; solvent B, 20% solvent A mixed with 80% acetonitrile. All of the solvents used were of HPLC grade. The elution program was described by Ibern-Gómez et al. (*37*).

Statistics. Significant differences among trials were assessed with two-sample analysis using Statgraphics 7.0.

2064 *J. Agric. Food Chem.,* Vol. 50, No. 7, 2002
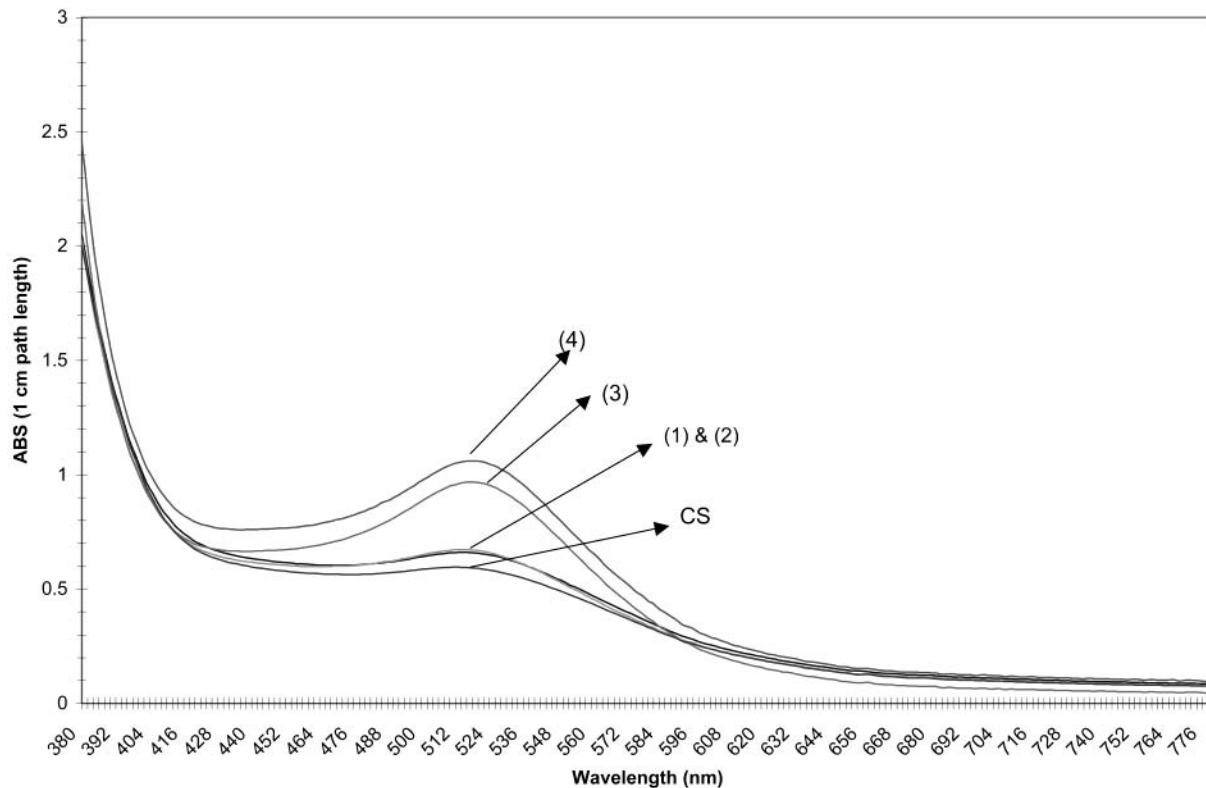
Darias-Martín et al.

Figure 3. Visible spectra of cv. Negramoll wines after alcoholic fermentation: CS, control; (1) 120 mg/L; (2) 240 mg/L; (3) 480 mg/L; (4) 960 mg/L.

Table 1. Mean Values of Color Enhancement at the Different Dosages of Caffeic Acid

date and dosage of caffeic acid	cv. Listán negro enhancement $(A - A_0)/A_0{}^a$ (%) on 520 nm	at 420 nm	cv. Negramoll enhancement $(A - A_0)/A_0{}^a$ (%) on 520 nm	at 420 nm	significance level on 520 nm[b]
at the end of alcoholic fermentation					
120 mg/L	25.4	7.2	12.7	8.5	*
240 mg/L	36.6	13.6	14.8	4.7	*
480 mg/L	40.5	14.4	62.9	7.7	*
960 mg/L	44.8	18.5	74.9	15.3	**
30th day after alcoholic fermentation					
120 mg/L	22.6	6.8	14.4	1.0	*
240 mg/L	34.1	13.6	18.8	2.8	*
480 mg/L	40.7	16.7	67.3	8.0	*
960 mg/L	40.7	17.4	89.2	19.5	*
90th day after alcoholic fermentation					
120 mg/L	8.8	4.3	36	10.4	*
240 mg/L	19.6	10.0	36	1.8	*
480 mg/L	35.4	17.5	94	16.9	**
960 mg/L	46.8	19.5	102	20.7	*
240th day after alcoholic fermentation					
120 mg/L	30.1	14.5	33.0	5.0	ns
240 mg/L	47.8	23.9	45.7	5.0	ns
480 mg/L	70.6	29.9	100.6	19.0	ns
960 mg/L	80.5	30.3	111.1	21.0	ns

^{a} A and A_0 are the absorbance in the presence and absence of caffeic acid, respectively. ^{b} Significance at which means differ as shown by two-sample analysis; *, **, denote significances at $p < 0.05$ and $p < 0.01$ respectively. ns, not significant.

RESULTS AND DISCUSSION

Effect on the Absorption Spectrum of Adding Caffeic Acid. Figures 2 and **3** show the increases in absorbance in the visible band produced by the addition of caffeic acid to each cultivar. These curves correspond to the first reading carried out when the wines had just finished the alcoholic fermentation. **Table 1** shows the color increase around 520 nm and at 420 nm and its variation with time. It can be seen that the effect produced by caffeic acid is significantly different for each

Table 2. Wavelength at the Maximum Absorbance Peak

dosage and date	λ_{max} (nm) cv. Listán negro	cv. Negramoll
at the end of alcoholic fermentation		
control	532	512
120 mg/L	532	514
240 mg/L	530	514
480 mg/L	528	518
960 mg/L	526	518
30th day after alcoholic fermentation		
control	532	514
120 mg/L	530	514
240 mg/L	530	514
480 mg/L	528	516
960 mg/L	526	516
90th day after alcoholic fermentation		
control	528	510
120 mg/L	524	512
240 mg/L	524	512
480 mg/L	522	514
960 mg/L	520	514
240th day after alcoholic fermentation		
control	522	508
120 mg/L	522	510
240 mg/L	520	512
480 mg/L	520	512
960 mg/L	520	512

cultivar in the first three readings, whereas in the last there is no significant difference. The highest doses of caffeic acid (480 and 960 mg/L) achieved a greater effect on cv. Negramoll. In general, the effect of this acid was greater on Negramoll, the cultivar that presented less color density.

In our study, the color increase was even more enhanced through time (**Table 1**). At ∼240 days after the end of alcoholic fermentation, the color enhancement varied between 30 and 111%. These results suggest that the initial combination between caffeic acid and anthocyanins could be a first step so that other

Effect of Caffeic Acid on Red Wine Color

J. Agric. Food Chem., Vol. 50, No. 7, 2002 **2065**

Table 3. Mean Values of Hue and Color Density

date and dosage of caffeic acid	cv. Listán negro		cv. Negramoll	
	E_{420}/E_{520}	$E_{420} + E_{520}$	E_{420}/E_{520}	$E_{420} + E_{520}$
at the end of alcoholic fermentation				
control	0.72	3.15	1.12	1.23
120 mg/L	0.61	3.71	1.07	1.36
240 mg/L	0.60	4.00	1.01	1.35
480 mg/L	0.59	4.10	0.74	1.65
960 mg/L	0.59	4.21	0.74	1.77
30th day after alcoholic fermentation				
control	0.73	3.10	1.10	1.08
120 mg/L	0.63	3.65	0.97	1.16
240 mg/L	0.61	3.95	0.95	1.19
480 mg/L	0.60	4.11	0.71	1.47
960 mg/L	0.60	4.12	0.69	1.65
90th day after alcoholic fermentation				
control	0.8	2.84	1.05	1.04
120 mg/L	0.76	3.03	0.85	1.27
240 mg/L	0.73	3.27	0.78	1.22
480 mg/L	0.69	3.62	0.63	1.60
960 mg/L	0.65	3.82	0.62	1.66
240th day after alcoholic fermentation				
control	0.86	2.53	0.97	1.02
120 mg/L	0.76	3.11	0.77	1.20
240 mg/L	0.72	3.46	0.71	1.26
480 mg/L	0.66	3.84	0.58	1.62
960 mg/L	0.62	3.98	0.57	1.67

types of reactions take place, forming more time-stable pigments during wine aging.

In general, the greater the dose of caffeic acid added, the greater the increase in color attained. The significant differences were, however, established as a whole between the lowest (120 and 240 mg/L) and highest (480 and 960 mg/L) doses.

It should be emphasized that the effect produced by caffeic acid not only involves an increase in absorbance around 520 nm but also, in general, in the whole spectrum including 420 nm. However, the greatest increases are around 520 nm due to which the hue index (E_{420}/E_{520}) diminishes when the dose of caffeic acid increases.

Table 2 shows the wavelengths with the maximum absorbance peaks (λ_{max}) in the visible spectrum for each wine. The control samples of cv. Listán negro initially have a peak

absorption at 532 nm and those for cv. Negramoll at 512 nm. These values varied through time and were 522 and 508 nm, respectively, after 240 days.

In trials carried out with model solutions with various copigments (*12, 17, 18, 20*), the copigmentation reaction produced a bathochromic shift in the maximum absorbance. In our study, a displacement of the maximum is observed, its magnitude depending on the concentration of the caffeic acid added: the greater the dose, the more displacement, up to $\Delta\lambda = 8$ nm.

However, this effect is different in each case in its direction of displacement. In cv. Listán negro the maximum is displaced toward lower values of λ (hypsochromic shift), only in cv. Negramoll is there a light displacement toward higher values (bathochromic shift). As the wines aged with time the magnitude of the displacement diminished and λ_{max} was displaced in all cases toward lower values (hypsochromically).

The mean tonality E_{420}/E_{520} and color density values ($E_{420} + E_{520}$) were obtained from the spectra as shown in **Table 3**. As expected, there is a decrease in color density in all of the wines as time progresses. However, for the time assayed, the tonality rises slightly with time in cv. Listán negro wines and drops in cv. Negramoll wines.

If the trends through time for the different trials are compared, the color density of the control samples is reduced by a greater percentage than those treated with caffeic acid. Thus, for the time span tested, this decrease is 20% for the cv. Listán negro control sample and only 3% for the added sample of 960 mg/L caffeic acid. In cv. Negramoll, these figures were 17 and 5%, respectively.

Tonality was decreased as the added caffeic acid dose was increased, this trend continuing through time.

These data clearly demonstrate color stabilization and protection against oxidation of caffeic acid in the red wines under trial.

HPLC Results. In an attempt to demonstrate the formation of new pigments between caffeic acid and the anthocyanins, chromatograms were run 240 days after alcoholic fermentation ended. At the highest doses of caffeic acid, the presence of pigments 1 and 2 at 520 nm in cv. Listán negro (**Figure 4**) and of pigment 2 in cv. Negramoll was observed. **Figure 5** shows
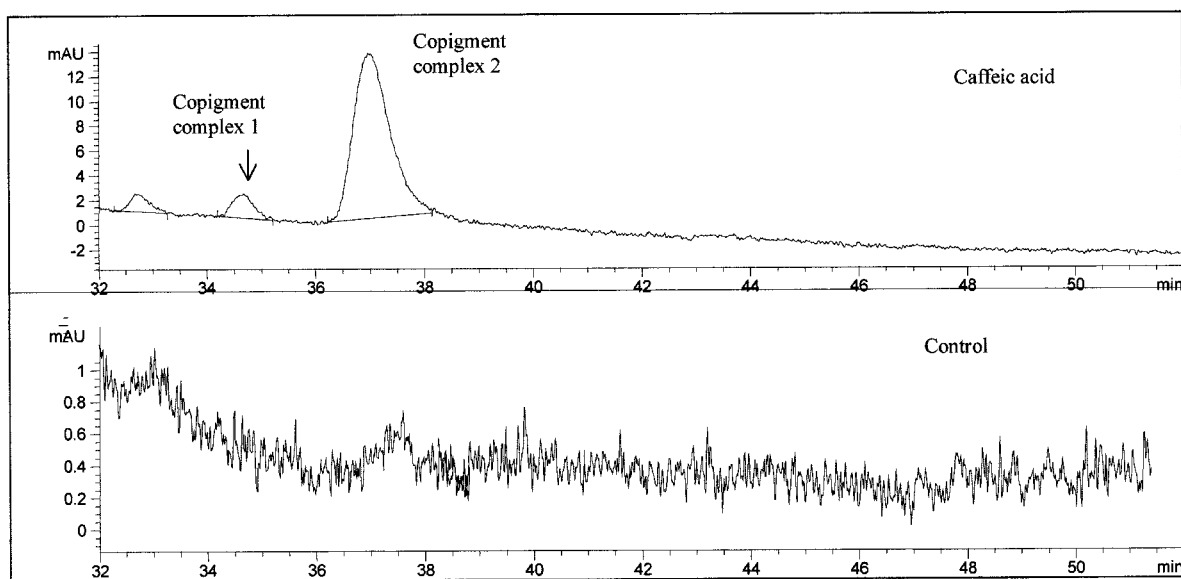

Figure 4. HPLC chromatograms of wines monitored at 520 nm: (A, top) sample cv. Listán negro with caffeic acid addition (960 mg/L); (B, bottom) control.
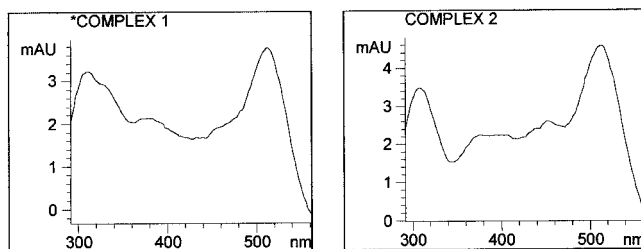
2066 *J. Agric. Food Chem.,* Vol. 50, No. 7, 2002

Darias-Martín et al.

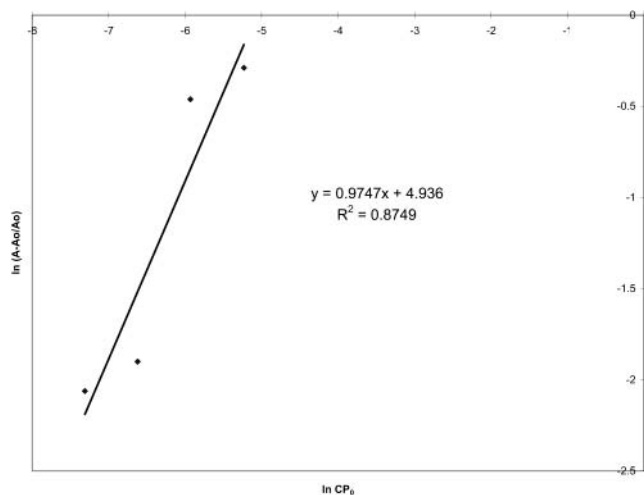**Figure 5.** Absorption spectra of pigments 1 and 2.

Figure 6. Plot of mean values of $\ln(A - A_0/A_0)$ vs $\ln(CP)_0$, cv. Negramoll wine.

the absorption spectra of these pigments, peaks being visible in the zone of anthocyanins (520 nm) and caffeic acid (320 nm). Adding more caffeic acid increases the area for the pigments.

Posssible Mechanism for the Copigmentation Reaction between Caffeic Acid and the Anthocyanins. Brouillard et al. in 1989 (*17*) established the equation $\ln(A - A_0/A_0) = \ln(Kr_1) + n \ln [CP]_0$, where A and A_0 are the absorbance at the maximum absorption in the visible, in the presence and absence of copigment, respectively; $[CP]_0$ is the added copigment concentration; r_1 represents the ratio between the molar absorption coefficient of the flavylium cation and the copigment complex; and K is the complexation constant.

The graph of added copigment concentration $\ln [CP]_0$ against the relative increase in the maximum absorption $\ln(A - A_0/A_0)$ in model solutions gives a straight line with slope n corresponding to the number of copigment molecules that form a complex with one flavylium cation. Its ordinate at the origin is proportional to the stability constant of this complex.

This mathematical model was applied to our data, considering it as an approximation because the model proposed by Brouillard fits model solutions and we have applied it to wines, where the governing equilibria are numerous, less well-known, and associated with aging processes. The data corresponding to the first register carried out at the end of alcoholic fermentation show approximately linear behavior, with a slope around 1 and an intercept of ~5 for cv. Negramoll and 3.5−4 for cv. Listán negro. The best approximation to linearity was that of Negramoll (**Figure 6**).

As the wines aged, these results varied and the graph deviated from linear to polynomial, due probably to the formation of new complexes and polymers during aging.

These preliminary values point toward the idea of an initial complex being formed between caffeic acid and anthocyanins in a ratio of 1:1.

Conclusions. Prior to this work, the copigment effect had always been estimated in model wine solution. In this study carried out during the production and maturation of red wines from two cultivars, we have demonstrated the importance of composition in grapes and must of nonpigmented compounds, such as caffeic acid, in color extraction during maceration and its later retention. Caffeic acid has produced greater color extraction compared to controls, in all trials. With time, it has exerted a color-stabilizing, oxidation-resisting effect. These results indicate that the copigment content of the grapes may be just as important in color extraction and retention as the extraction techniques used in cellars.

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Effect of Caffeic Acid on Red Wine Color

J. Agric. Food Chem., Vol. 50, No. 7, 2002 **2067**

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