Effect of Storage Temperature and Pyruvate on Kinetics of Anthocyanin Degradation, Vitisin A Derivative Formation, and Color Characteristics of Model Solutions

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The formation of vitisin A, an anthocyanin formed naturally in small quantities in maturing port wines, was studied in model wine solutions at several storage temperatures (10, 15, 20, and 32 °C). Vitisin A was formed through the interaction between malvidin 3-glucoside and pyruvic acid, Acylated forms of vitisin A, having the 6-position of the sugar acylated with acetic acid (3-acetylvitisin A) and *p*-coumaric acid (3-*p*-coumarylvitisin A), were also formed through the interaction between pyruvic acid and malvidin 3-acetylglucoside and malvidin 3-*p*-coumarylglucoside, respectively. A maximum degradation of the anthocyanins was obtained at higher temperatures, and it followed a first-order kinetics both with and without pyruvic acid in the solution. Whereas at low temperatures (10 and 15 °C) the presence of pyruvic acid accelerated the kinetic reaction, at higher temperatures (20 and 32 °C) it decreased it. The activation energy values for the degradation of the three anthocyanins in model solutions without and with pyruvic acid were not significantly different from each other. At low temperatures the highest concentrations of vitisin A compounds were obtained. All solutions showed a decrease in *L** value, indicating that all solutions became darker. This change increased with increasing temperature. All model solutions increased in the hue angle, indicating that the solutions changed from a bluish-red to an orange-red or even brownish-red color. Samples without pyruvic acid remained lighter and became browner than those with pyruvic acid. A good correlation between the amount of vitisin A in the solution and hue angle was found, indicating that vitisin A may contribute the orange-red of solutions, compared to the browner control.

Keywords: *Model wine solutions; anthocyanins; pyruvic acid; temperature; vitisin A*

INTRODUCTION

Anthocyanins are significant components of red wine color, which is a major attribute of wine quality. The influence of processing variables on the color quality of red wines and the effect of color on overall quality has been demonstrated (Timberlake and Bridle, 1976). The color changes in red wines due to condensation reactions between anthocyanins and other phenolic compounds naturally occurring in wines are well-documented, and until recently these were the reactions thought to exert the greatest influence on the color changes in red wines during maturation.

Earlier analyses of red port wine pigments revealed a number of unidentified peaks, eluting soon after malvidin 3-glucoside (Somers, 1966; Bakker, 1985; Dallas and Laureano, 1994). Properties of one of these anthocyanins included resistance to color bleaching by sulfur dioxide. This stability in the presence of sulfur dioxide is consistent, by analogy with flavylium salt studies (Timberlake, 1968; Timberlake and Bridle, 1968), with the presence of a 4-substituent on the anthocyanin molecule. Bakker et al. (1997) reported the isolation of this anthocyanin-type pigment, named vitisin A, suggesting that vitisin A is based on malvidin 3-glucoside with a 4-substitution of $C_3H_2O_2$ by linkage with the oxygen from the 5-hydroxyl.

Recent work in our laboratory (Romero and Bakker, 1999) has revealed, in model wine solution, the formation of these new malvidin-derived compounds, which were synthesized in reaction of malvidin 3-glucoside with pyruvic acid. The extent to which these reactions occur was found to depend on anthocyanin composition, pH, and pyruvic acid concentration, with generally the highest amount of vitisins formed at pH 3 and high concentrations of pyruvic acid. We also reported the presence in these model studies of vitisin A derived from malvidin 3-glucoside, in addition to acetylvitisin A and 3-*p*-coumarylvitisin A, derived from malvidin 3-acetylglucoside and 3-*p*-coumarylglucoside, respectively. Fulcrand et al. (1998) also reported the formation of pigments generated by reaction between anthocyanins and pyruvic acid, but using reaction conditions less similar to those of wine.

The formation of vitisin A as a result of a reaction between malvidin 3-glucoside and pyruvic acid is in agreement with our observations that these compounds are more abundant in fortified port wine than in red table wine (Bakker, 1985). Port wine is made by adding fortifying grape spirit to stop the fermentation halfway through, thus maintaining natural sweetness (Reader and Dominguez, 1995), but at this stage the pyruvic acid concentration is also thought to be higher in the

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fermenting wine than when the fermentation is allowed to continue to dryness (Whiting and Coggins, 1960).

Many factors during processing and storage of wine have been shown to influence degradation of anthocyanins, such as temperature, pH, phenolic compounds, sugar and sugar degradation products, oxygen, and ascorbic acid (Markakis, 1982; Mazza and Brouillard, 1987). Anthocyanins are generally reported to be unstable in model systems stored at increased temperatures (Markakis, 1982). Baranowski and Nagel (1983) observed in a model wine system that storage temperature affected malvidin 3-glucoside degradation and polymer formation. At 42 °C the greatest amount of polymeric material was present, but the polymers were more stable to degradation and precipitation when formed at 32 °C or less.

The color properties of the vitisins are expected to influence the red wine color. The hypsochromic spectral shifts of vitisin A from malvidin 3-glucoside indicate a shift to orange-brown (Bakker and Timberlake, 1997). Indeed, in model wine solutions, we found that vitisin A expressed about 11 times (pH 3) or 14 times (pH 2) more color than the normal anthocyanins (Romero and Bakker, 1999). During aging, the changes in the red color of the wine are attributed to different reactions, involving anthocyanins, flavanols and flavonols, oxygen, aldehydes, etc. The bluish-red color, attributable to the main anthocyanins present, changes to a slightly more orange red, attributable to the vitisin A compound (Romero and Bakker, 1999).

Our objective was to determine the kinetics of the degradation of the main anthocyanins in model solutions at a range of temperatures, to determine the formation of vitisin A compounds through the reaction between malvidin 3-glucoside, malvidin 3-acetylglucoside, malvidin 3-*p*-coumarylglucoside, and pyruvic acid. Kinetic parameters such as reaction order, reaction rate constants, and activation energies were determined to define degradation reactions. Additionally, the effect of vitisin A formation at different temperatures on the color changes of these model wines was evaluated.

MATERIALS AND METHODS

Chemicals. Pyruvic acid was purchased from Sigma (St. Louis, MO). Solvents were HPLC-grade. Anthocyanins were extracted from Touriga nacional grape skins (Douro Valley, Portugal) with methanol containing 3% (v/v) formic acid. The methanolic solution was concentrated by rotary evaporator (Büchi) at 30 °C and dried under vacuum. The grape skin extract was kept in the desiccator until it was used.

Model Solutions. A potassium hydrogen tartrate (Sigma, Dorset, U.K.) buffer (0.02 M, pH 3.5) containing 10% ethanol (v/v) was used as a model wine base (Bakker et al., 1993). The dry grape skin extract was dissolved in this buffer solution to study the effect of temperature on the formation of vitisin A from the reaction between malvidin 3-glucoside and pyruvic acid. Different concentrations of pyruvic acid were added, to obtain molar ratios of pyruvic acid to total anthocyanins (PA/TA) between 0 (no added pyruvic acid) and 300 units. Finally, the pH of all solutions was adjusted to pH 3.7 by adding Na_2CO_3 (Sigma).

The experiment was carried out at 10, 15, 20, and 32 °C. All solutions were filtered, and duplicate samples (30-mL sample in a 50-mL stopped vial) were incubated in the dark in the presence of air.

Samples from separate vials were analyzed periodically by HPLC and spectrophotometry.

Anthocyanins Analysis. Samples were analyzed by HPLC on a Hewlett-Packard (Germany) 1090 M Series II chromatograph with auto injector (25 μL). A Hypersil ODS column, 2.1 mm × 100 mm (5 μm), was used at 40 °C, with diode array detection at 520 nm. The elution conditions and anthocyanin quantification had been explained in a previous publication (Romero and Bakker, 1999). The data of each measurement are the average of duplicate samples.

Pyruvic Acid Analysis. This acid was determined at time zero by an enzymatic method (Sigma, food analysis enzymatic kit, St. Louis, MO).

Colorimetric Measurements. Absorption spectra by scanning solutions in 1-mm path length glass cells from 380 to 770 nm were carried out using a Philips PU8740 UV−vis spectrophotometer, equipped with a computer software program to calculate tint, color density, and CIELAB 76 parameters (L^*, a^*, b^*, chroma, and hue angle). Tint is defined as the ratio (A_{420nm}/A_{520nm}) and color density as the sum ($A_{520nm} + A_{420nm}$); higher values for darker wines result in higher color density values, whereas an increase in brownness relative to redness increases the tint value. L^*, a^*, and b^* values describe a three-dimensional color space. The vertical axis L^* is a measure of lightness, from completely opaque (0) to completely transparent (100), whereas on the hue-circle a^* is a measure of redness (or $-a^*$ of greenness) and b^* of yellowness (or $-b^*$ of blueness). The hue angle (degrees) expresses the color nuance and is calculated from $h = \text{arctg}(b^*/a^*)$ (McLaren, 1980); when the angle increases from 0° to 60°, the color changes from violet-red through red to reddish-brown. The chroma (c) was obtained as $(a^{*2} + b^{*2})^{1/2}$. The data of each measurement are the average of duplicate samples.

Statistical Analysis. Linear regression analysis of variance and standard deviation were performed using SPSS.

RESULTS AND DISCUSSION

Degradation of Anthocyanins in Model Solutions. Model solutions were stored at several temperatures (10, 15, 20, and 32 °C) in darkness. They included a grape extract from the variety Touriga nacional, in which an amount of 19.17 ± 5.46 mg/L of malvidin 3-glucoside was present the first day, followed by malvidin 3-*p*-coumarylglucoside (11.37 ± 3.08 mg/L), malvidin 3-acetylglucoside (4.42 ± 2.26 mg/L), and a small amount of vitisin A derivatives, 0.67 ± 0.20 mg/L of vitisin A and 0.40 ± 0.26 mg/L of *p*-coumarylvitisin A. It was evident that during the period of study, the concentrations of these anthocyanins decreased.

Our results confirmed that anthocyanin degradation reaction could be modeled by first-order kinetics, in agreement with previous reports (Bakker et al., 1993; Cemeroglu et al., 1994; Romero and Bakker, 1999). Using linear regression to plot the ln of the anthocyanin concentration at various storage times, the reaction rate constants (k) were determined from the slopes (Table 1). A very high correlation (r^2) was observed between the ln of the three main anthocyanin concentrations (malvidin 3-glucoside, malvidin 3-acetylglucoside, and malvidin 3-*p*-coumarylglucoside) with storage time at all temperatures assayed (Table 1). The presence of pyruvic acid in the solutions did not affect the order of the reaction, as was previously shown by Romero and Bakker (1999).

Analysis of variance on the reaction rate constants (k) showed highly significant effects of pyruvic acid addition, temperature, and anthocyanin (Table 2). Overall, the mean loss of anthocyanins is significantly lower in the presence of pyruvic acid (Table 3). As expected, loss of anthocyanins in model wine solutions significantly increased with increasing storage temperature (Table 3), although there was no significant difference between 10 and 15 °C. At higher temperatures we observed the formation of some brown precipitates,

Table 1. Reaction Rates and Activation Energies (E_a) for Disappearance of the Three Main Anthocyanins in Model Systems[a]

anthocyanin	PA/TA	temp (°C)	k (days^{-1})	n	lower bound (95%)	upper bound (95%)	E_a (kJ/mol)	lower bound (95%)	upper bound (95%)
malvidin 3-glucoside	0	10	−0.007(0.975)[b]	10	−0.008	−0.006	1.327	−0.096	2.750
		15	−0.008(0.963)	10	−0.010	−0.007			
		20	−0.042(0.984)	9	−0.047	−0.037			
		32	−0.085(0.984)	8	−0.098	−0.073			
	300	10	−0.007(0.986)	10	−0.008	−0.007	1.019	0.356	1.682
		15	−0.012(0.987)	10	−0.013	−0.011			
		20	−0.029(0.981)	9	−0.032	−0.026			
		32	−0.057(0.975)	8	−0.065	−0.049			
malvidin 3-ac-glucoside	0	10	−0.007(0.973)	10	−0.008	−0.006	1.303	−0.440	3.046
		15	−0.008(0.919)	9	−0.010	−0.006			
		20	−0.049(0.994)	7	−0.054	−0.043			
		32	−0.079(0.933)	6	−0.108	−0.049			
	300	10	−0.008(0.875)	10	−0.010	−0.005	0.948	0.439	1.458
		15	−0.017(0.933)	9	−0.023	−0.012			
		20	−0.026(0.921)	7	−0.032	−0.020			
		32	−0.060(0.927)	6	−0.079	−0.040			
malvidin 3-*p*-c-glucoside	0	10	−0.008(0.906)	10	−0.010	−0.006	1.557	−0.204	3.318
		15	−0.012(0.933)	10	−0.014	−0.009			
		20	−0.077(0.997)	8	−0.083	−0.071			
		32	−0.151(0.984)	6	−0.210	−0.093			
	300	10	−0.008(0.925)	10	−0.010	−0.006	1.253	0.626	1.880
		15	−0.015(0.938)	10	−0.017	−0.011			
		20	−0.038(0.927)	8	−0.046	−0.029			
		32	−0.097(0.963)	6	−0.123	−0.070			

[a] Values of molar ratio PA/TA were 0 and 300 units. The experiment was carried out at pH 3.7 and four temperatures (10, 15, 20, and 32 °C). [b] Values of r^2.

Table 2. Analysis of Variance on the Reaction Rate Constants for Disappearance of the Three Main Anthocyanins in Model Systems[a]

source	df	mean square	F	signif[b]
pyruvic acid	1	0.0011	24.810	0.002
temp	3	0.0083	195.628	0.000
anthocyanin	2	0.0010	23.765	0.001
pyruvic acid × temp	3	0.0005	12.774	0.005
pyruvic acid × anthocyanin	2	0.0001	3.041	0.122
temp × anthocyanin	6	0.0004	9.587	0.007

[a] Values of molar ratio PA/TA were 0 and 300 units. The experiment was carried out at pH 3.7 and four temperatures (10, 15, 20, and 32 °C). [b] $\alpha = 0.05$.

Table 3. Mean Reaction Rate constants (k) for Anthocyanin Degradation As Affected by Pyruvic Acid, Temperature, and Malvidin Derivative[a]

source	mean of k[b] (days^{-1})	lower bound (95%)	upper bound (95%)
pyruvic acid			
PA/TA = 0	−0.0444 a	−0.0490	−0.0398
PA/TA = 300	−0.0311 b	−0.0358	−0.0266
temp (°C)			
10	−0.0075 a	−0.0140	−0.0010
15	−0.0120 a	−0.0185	−0.0055
20	−0.0435 b	−0.0500	−0.0370
32	−0.0882 c	−0.0947	−0.0817
anthocyanin			
malvidin 3-glucoside	−0.0309 a	−0.0365	−0.0252
malvidin 3-acetylglucoside	−0.0318 a	−0.0374	−0.0261
malvidin 3-*p*-coumarylglucoside	−0.0508 b	−0.0564	−0.0451

[a] The experiment was carried out at pH 3.7. [b] No letter in common within each factor denotes a significant difference of at least a 0.05 level.

which could be attributed to an irreversible change caused by anthocyanin degradation due to storage at a higher temperatures (Brouillard and Dubois, 1977). Regarding the anthocyanins, the degradation of malvidin 3-*p*-coumarylglucoside was significantly faster than the corresponding degradation of malvidin 3-glucoside and malvidin 3-acetylglucoside, there being no difference between the latter two anthocyanins (Table 3). These observations agree with Bakker (1986), who reported in port wine a faster loss of malvidin 3-*p*-coumarylglucoside than of malvidin 3-glucoside. Likewise, this author reported in port wines faster losses of malvidin 3-acetylglucoside than of malvidin 3-glucoside, which was not confirmed in our model wines.

There was no significant interaction between pyruvic acid and compound, but there was interaction between temperature and anthocyanin and between temperature and pyruvic acid addition (Table 2). The effect of pyruvic acid on the k value was dependent on the temperature (Table 1). The degradation of malvidin 3-glucoside, malvidin 3-acetylglucoside, and malvidin 3-*p*-coumarylglucoside was faster at low temperatures (10 and 15 °C) in the presence of pyruvic acid, but at high temperatures (20 and 32 °C) the opposite occurred: higher k values were found in the absence of pyruvic acid. We do not have any explanation for this effect.

Activation energies (E_a) (Table 1) were determined by the Arrhenius equation for anthocyanin degradation (Exner, 1988). The activation energy was higher in the absence of pyruvic acid for all three anthocyanins, but the confidence interval of activation energy for each anthocyanin degraded in the presence of pyruvic acid overlaps in part the confidence interval of activation energy of its respective control. This means that there are no statistical differences ($\alpha = 0.05$) between the E_a values obtained in absence and presence of pyruvic acid for the three monomeric anthocyanins. Baranowski and Nagel (1983) reported a comparable E_a value of 1.7342 kJ/mol for malvidin 3-glucoside in model wines, using different experimental conditions.

Formation of New Compounds. The interaction between malvidin 3-glucoside and pyruvic acid caused the formation of a new pigment, called vitisin A, previously isolated and identified from red wines (Bakker and Timberlake, 1997) and from model wine solutions

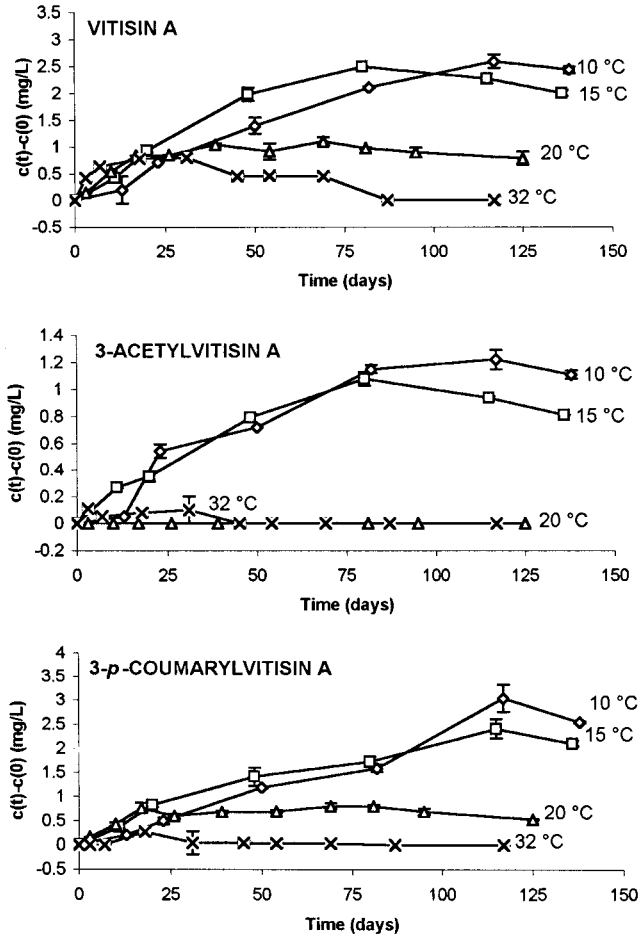

Figure 1. Increase of vitisin A, 3-acetylvitisin A, and 3-*p*-coumarylvitisin A concentration in model wines at pH 3.7 in the presence of pyruvic acid (PA/TA = 300 units), during 140 days at 10, 15, 20, and 32 °C. Each point is the average of two measurements and where error bars are not visible, determinations are within the size of the symbols on the graph.

(Romero and Bakker, 1999). Likewise, the acylated forms, having the 6-position of the sugar acylated with acetic acid (3-acetylvitisin A) and *p*-coumaric acid (3-*p*-coumarylvitisin A), formed through the interaction between pyruvic acid and malvidin 3-acetylglucoside and malvidin 3-*p*-coumarylglucoside, respectively, were formed in these model solutions, confirming previous reports (Romero and Bakker, 1999).

Figure 1 shows the evolution of these new compounds during 140 days of storage, at four temperatures (10, 15, 20, and 32 °C). Generally, more vitisin A type compounds formed at the lower temperatures. Vitisin A was formed most rapidly at 32 °C, but after only 31 days, its concentration started to decrease. The degradation of vitisin A compounds appeared more rapid at higher than at lower temperatures. The increase of vitisin A concentration was slower at low storage temperature, rising a maximum value at 15 °C, after 80 days, and at 10 °C, after 232 days (data not shown). The other two new compounds (3-acetylvitisin A and 3-*p*-coumarylvitisin A) are formed similarly at low temperatures, but very little if any formed at higher temperatures (Figure 1).

The loss of the three main anthocyanins is associated with the formation of the new compounds (Table 4). At the end of each experiment, which lasted longer at the lower temperature, between 79 and 100% of the anthocyanins were lost, coinciding with the formation of up to 27% vitisin A compounds. Independent of temperature, we found for the ratio [vitisin A compound formed (%)/anthocyanin loss (%)] bigger values for the pair malvidin 3-*p*-coumarylglucoside with 3-*p*-coumarylvitisin A than for the pair malvidin 3-glucoside with vitisin A (Table 4). Thus, more 3-*p*-coumarylvitisin A is formed than vitisin A for comparable losses of their respective precursors, malvidin 3-*p*-coumarylglucoside and malvidin 3-glucoside. However, it must be kept in mind that other reactions involving anthocyanins may also occur.

Influence of Temperature on the Color Solutions. The CIELAB 76 measurements give color information closely associated to human vision (McLaren, 1980), using data collected over the entire visible range of wavelengths. However, using the entire data set value, there were good linear correlations between tint and hue angle at each temperature ($r^2 = 0.893$, $n = 20$ at 10 °C; $r^2 = 0.989$, $n = 20$ at 15 °C; $r^2 = 0.990$, $n = 20$ at 20 °C; $r^2 = 0.969$, $n = 22$ at 32 °C) and between color density and L^* ($r^2 = 0.997$, $n = 20$ at 10 °C; $r^2 = 0.996$, $n = 20$ at 15 °C; $r^2 = 0.950$, $n = 20$ at 20 °C; $r^2 = 0.637$, $n = 22$ at 32 °C). The results here provide support that tint and color density, traditionally used for wine color assessments, compare well with CIELAB 76 measurements in these model systems, confirming previous observations (Romero and Bakker, 1999). Only the CIELAB 76 data will be discussed for this paper.

Considerable changes in L^* values (Figure 2) and hue angles (Figure 3) occurred at all temperatures, with greater differences between samples with and without pyruvic acid developing at higher storage temperature. These results showed that storage temperature affects the color development in the solutions, which is expected to relate with the anthocyanin composition. Particularly, Van Buren et al. (1974) showed a relation between L^* and malvidin 3-glucoside evolution, finding a decrease of L^* with an increase of malvidin 3-glucoside concentration on the model wine solution.

L^* value decreased in all solutions, indicating that the solutions became darker with the storage time (Figure 2), in particular when pyruvic acid was present. Without pyruvic acid (Figure 2a), the decrease in L^* value was very small even after 175 days of storage, although it was greatest at 32 °C, showing a straight line ($r^2 = 0.90$). In the presence of pyruvic acid in the solutions (Figure 2b), the decrease in L^* values is similar for 10 and 15 °C, being possible to observe a linear correlation between decrease of L^* values and storage time at 20 °C ($r^2 = 0.92$) and 32 °C ($r^2 = 0.80$), with the greatest slope at the highest temperature. At 32 °C a faster loss of anthocyanins (Table 1), including vitisin A compounds (Figure 1), was observed, possibly leading to a faster formation of polymeric anthocyanins giving a darker solution. Similar behavior was observed by Gao et al. (1997) when they studied the influence of fermentation temperature on wine color and reported that a fermentation temperature of 30 °C favored both the formation of polymeric anthocyanins and a darker wine.

Generally, linear correlations were observed between increases in hue angle and storage time, showing that during storage all samples changed from a purplish-red toward brown-red (Figure 3). Some exceptions were found, including a high temperature in the absence of pyruvic acid (Figure 3a), it being not possible to obtain a simple correlation at 20 and 32 °C. Pyruvic acid affected the slopes at each temperature, with the slopes

Table 4. % Loss (−) of Malvidin Anthocyanins and % Increase (+) in Vitisin A Compounds, Calculated at the Time of Maximum Vitisin A Compound Concentration[a]

T (°C)	time (days)	Mv 3-Glu (%)	Vit A (%)	Vit A/Mv 3-Glu	Mv 3-AcGlu (%)	3-AcVit A (%)	3-AcVit A/Mv 3-AcGlu	Mv 3-*p*-CmGlu (%)	3-*p*-CmVit A (%)	3-*p*-CmVit A/Mv 3-*p*-CmGlu
10	232	−83.27(0.38)[b]	+18.45(0.30)	0.22	−87.65(0.09)	+27.45(0.03)	0.31	−88.46(0.07)	+25.04(0.32)	0.28
15	80	−89.21(0.59)	+13.32(0.35)	0.15	−100.0(3.28)	+16.77(0.04)	0.17	−92.76(1.83)	+21.33(0.16)	0.23
20	69	−90.27(0.25)	+8.09(0.13)	0.09	−79.78(0.04)	+0.00(0.00)	0.00	−94.91(0.13)	+11.63(0.08)	0.12
32	31	−91.18(0.21)	+3.14(0.11)	0.03	−93.75(0.00)	+2.23(0.14)	0.02	−89.72(0.00)	+5.45(0.07)	0.06

[a] Model solution contained extract of grape and pyruvic acid with a value of 300 units for the molar ratio PA/TA. Amount of new anthocyanins refers to the initial concentration of the anthocyanin coming from (i.e., Vit A (%) = [Vit $A_{(t=\text{end days})}$ − Vit $A_{(t\ =\ 0\ \text{day})}$] × 100/Mv 3-$Glu_{(t=0\ \text{day})}$). Malvidin 3-glucoside (Mv 3-Glu), vitisin A (Vit A), Malvidin 3-acetylglucoside (Mv 3-AcGlu), 3-Acetylvitisin A (3-AcVit A), Malvidin 3-*p*-Coumarylglucoside (Mv 3-*p*-CmGlu), and 3-*p*-Coumarylvitisin A (3-*p*-CmVit A). [b] Standard deviation of the differences.

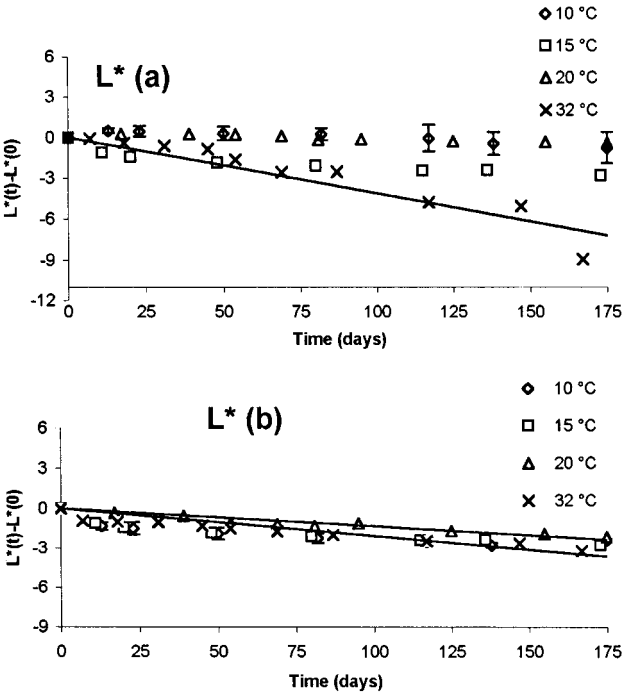

Figure 2. Decrease of *L** value in model wines at pH 3.7 in absence (a) and presence (b) of pyruvic acid (PA/TA = 300 units), during 175 days at 10, 15, 20, and 32 °C. (a) $y = -0.041x$ ($r^2 = 0.90$) at 32 °C. (b) $y = -0.014x$ ($r^2 = 0.92$) at 20 °C, and $y = -0.021x$ ($r^2 = 0.80$) at 32 °C. Each point is the average of two measurements and where error bars are not visible, determinations are within the size of the symbols on the graph.

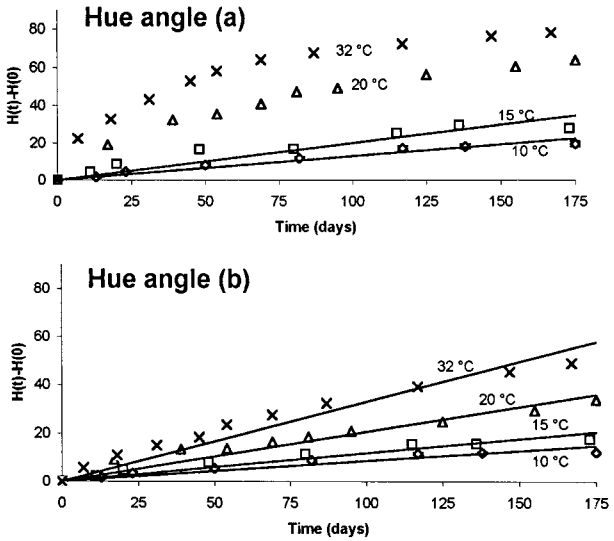

Figure 3. Increase of hue-angle (degrees) value in model wines at pH 3.7 units in absence (a) and presence (b) of pyruvic acid (PA/TA = 300 units), during 175 days at 10, 15, 20 and 32 °C. (a) $y = 0.13x$ ($r^2 = 0.95$) at 10 °C, $y = 0.20x$ ($r^2 = 0.85$) at 15 °C. (b) $y = 0.08x$ ($r^2 = 0.90$) at 10 °C, $y = 0.12x$ ($r^2 = 0.92$) at 15 °C, $y = 0.21x$ ($r^2 = 0.91$) at 20 °C, and $y = 0.33x$ ($r^2 = 0.92$) at 32 °C. Each point is the average of two measurements and where error bars are not visible, determinations are within the size of the symbols on the graph.

in model solutions that contained pyruvic acid increasing more slowly than in those without pyruvic acid. There were clear effects of temperature: the samples both with and without pyruvic acid increased more in hue angle with increased storage temperature. Picinelli et al. (1994), using similar model wine solutions, and Bakker et al. (1986), using port wines, also reported an increase in hue angle during aging. In our studies, the hue angle in the model without pyruvic acid at the end of the experiment ranged from 19.4° at 10 °C to 78.2° at 32 °C, whereas in the models with pyruvic acid they all were lower, ranging from 12.1 degrees to 49.0 degrees (Figure 3). Previously, Romero and Bakker (1999) studied the effect of pH on vitisin A formation in model wines, and found that the vitisin A compounds contributed redness to the stored solution, in comparison with browner controls without vitisin A compounds. The results of this current study also show that aging with the formation of vitisin A-type compounds tends to result in a redder model solution than aging without the formation of these compounds, clearly confirming their contribution to a reddish color.

Relation between Vitisin A Compounds and Color of Solutions. Romero and Bakker (1999) found a good correlation between the percentage of vitisin A of the total anthocyanins and the hue angle at different pH values. Such correlations are also observed in these studies on storage temperature (Table 5). When all the data at each temperature were combined, there were no significant correlations between percentage of vitisin A and hue angle, indicating that the changes in hue angle depend on the reaction conditions used.

Only weakly significant correlations were found for the control, but highly significant ones were found for the samples, all containing considerable vitisin A concentrations (Table 5). Thus it would seem that the vitisin A compounds are browner than the malvidin 3-glucoside and its acylated forms, but redder than more polymerized material, thus contributing an orange-redness to the solutions. This is in agreement with Bakker and Timberlake (1997), who reported pure vitisins to be browner than malvidin 3-glucoside.

However, the correlation between the sum of all vitisin A compounds formed and the hue angle was lower at 15 and 20 °C, and was not significant at 32 °C (Table 6), as compared to the correlations using vitisin A only (Table 5). Possibly there could be a difference in color quality among the three vitisins contributing. For

Table 5. Correlation (r^2) between the Vitisin A (%) with Hue Angle (Degrees)[a]

model solution	10 °C	15 °C	20 °C	32 °C
all data	NS ($n = 20$)	NS ($n = 20$)	NS ($n = 20$)	NS ($n = 20$)
PA/TA = 0	0.640[c] ($n = 10$)	0.477[c] ($n = 10$)	NS ($n = 10$)	0.667[c] ($n = 10$)
PA/TA = 300	0.843[b] ($n = 10$)	0.975[b] ($n = 10$)	0.968[b] ($n = 10$)	0.790[b] ($n = 10$)

[a] The model solution contained extract of grape and pyruvic acid in two different concentrations (PA/TA = 0 and 300 units). The experiments were carried out at pH 3.7 and four temperatures (10, 15, 20, and 32 °C). [b] $p \leq 0.001$. [c] $p \leq 0.05$. NS: Not significant, $p > 0.05$.

Table 6. Correlation (r^2) between the Sum of Vitisin A Compounds (%) with Hue Angle (Degrees)[a]

model solution	10 °C	15 °C	20 °C	32 °C
all data	NS ($n = 20$)	NS ($n = 20$)	NS ($n = 20$)	NS ($n = 20$)
PA/TA = 0	0.763[b] ($n = 10$)	NS ($n = 10$)	NS ($n = 10$)	NS ($n = 10$)
PA/TA = 300	0.976[b] ($n = 10$)	0.718[c] ($n = 10$)	0.905[b] ($n = 10$)	NS ($n = 10$)

[a] The model solution contained extract of grape and pyruvic acid in two different concentration (PA/TA = 0 and 300 units). The experiments were carried out at pH 3.7 and four temperatures (10, 15, 20 and 32 °C). [b] $p \leq 0.001$. [c] $p \leq 0.01$. NS: Not significant, $p > 0.05$.

example, Bakker and Timberlake (1997) reported that 3-acetylvitisin A exhibited a bathochromic shift from vitisin A, and Romero and Bakker (1999) showed a similar effect for 3-acetylvitisin A and 3-*p*-coumarylvitisin A from vitisin A.

CONCLUSIONS

The interaction between the main malvidin 3-glucoside in model wine solutions and added pyruvic acid led to the formation of a new anthocyanin, called vitisin A. The acylated forms, having the 6-position of the sugar acylated with acetic acid (3-acetylvitisin A) and *p*-coumaric acid (3-*p*-coumarylvitisin A), were formed through the interaction between pyruvic acid and malvidin 3-acetylglucoside and malvidin 3-*p*-coumarylglucoside, respectively. Four storage temperatures were evaluated (10, 15, 20, and 32 °C), and maximum degradation of the anthocyanins was obtained at higher temperatures. This degradation followed first-order kinetics both with and without pyruvic acid in the solution. The presence of this acid had two opposite effects on the degradation of anthocyanins: at low temperatures (10 and 15 °C) it accelerated the kinetic reaction, but at higher temperatures (20 and 32 °C) it decreased it. Presence of pyruvic acid in the solution had no significant effect on the activation energy values for the degradation of the three anthocyanins. At the end of the experiment, the highest concentrations of vitisin A compounds were determined in the models stored at low temperatures.

On the other hand, the color evolution was similar in all experiments; all solutions showed a decrease in L^* value, more rapid at higher temperatures, indicating that all solutions became darker with storage time. Likewise, an increase in the hue-angle value occurred in all solutions, leading a change from a purplish-red toward a brownish-red color. As expected, a higher storage temperature gave a larger increase in hue-angle value. Samples without pyruvic acid were lighter and browner than those with pyruvic acid. A good correlation between the amount of vitisin A in the solution and hue angle was found, indicating that vitisin A may contribute the orange-red of solutions, compared to the browner control.

Further investigations to study the influence of acetaldehyde, acids, etc., on the formation of these compounds in model systems will give a better understanding of the maturation processes in wines, important information that could be relevant to the storage of port wine to obtain a stable reddish color.

ABBREVIATIONS USED

PA/TA, molar ratio pyruvic acid/total anthocyanins; HPLC, high-performance liquid chromatography; UV−vis, ultraviolet−visible.

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