JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2005,** *53,* 49−56 **49**

Stability of Copigmented Anthocyanins and Ascorbic Acid in a Grape Juice Model System

CARMEN H. BRENES,*,† DAVID DEL POZO-INSFRAN,‡ AND STEPHEN T. TALCOTT‡

Department of Food Technology, ITESM-Campus Monterrey, E. Garza Sada 2501 Sur, C.P. 64849, Monterrey, N.L., Mexico, and Department of Food Science and Human Nutrition, University of Florida, P.O. Box 110370, Gainesville, Florida 32611-0370

The stability of red grape anthocyanins (*Vitis vinifera*) was evaluated in a model juice system during normal (25 °C) and accelerated storage (35 °C) in the presence of ascorbic acid. Rosemary polyphenolic cofactors (0, 0.2, and 0.4% v/v) were evaluated as anthocyanin stabilizing agents. Cofactor addition resulted in concentration-dependent hyperchromic (up to 178%) and bathochromic (up to 23 nm) shifts, indicating a more intense red coloration of the models. Anthocyanin and ascorbic acid degradation followed first-order kinetics during storage. Results showed that copigmented treatments underwent a lower conversion of L-ascorbic acid into dehydroascorbic acid during storage when compared to the control, favorably impacting the vitamin retention of these models. Copigmentation did not affect anthocyanin degradation in the absence of ascorbic acid but in its presence aided to retain a higher anthocyanin content than the control. This study indicated that the addition of anthocyanin cofactors could be used to reduce the pigment and vitamin degradation while masking detrimental color changes in anthocyanin containing products.

KEYWORDS: Anthocyanins; copigmentation; ascorbic acid; dehydroascorbic acid; stability

INTRODUCTION

A steady increase in the development of natural food colorants has been observed in recent years (*1*, *2*), not only due to consumer preferences for natural pigments but also encouraged by their health-related benefits (*3*−*6*). Anthocyanins are considered as potential replacements for synthetic dyes because of their bright, attractive colors and water solubility, which allows their incorporation into aqueous food systems (*2*). However, many limitations exist for their commercial application in food products due to high raw material costs and poor stability, which depends on their chemical structure, concentration, pH, temperature, oxygen, light, polymeric forms, and presence of cofactors and/or ascorbic acid (AA). The latter factor is the focus of the present study due to its negative impact on nutritional quality, functional properties, and color degradation in fruit products (*6*−*8*). A mechanism for the mutual degradation of anthocyanins and AA was proposed as a direct condensation between both compounds that causes accelerated loss of each phytochemical (*7*−*9*). Conversely, Garcia-Viguera et al. (*10*) in support of Iacobucci and Sweeny (*11*) proposed a free radical mechanism where cleavage of the pyrilium ring resulted as a consequence of oxidation initiated by activation of molecular oxygen induced by AA. Despite the proposed degradation mechanism, preventing the mutual degradation of both compounds is an aspect than can benefit both processors and consumers.

Anthocyanin copigmentation reactions are common in nature and result from the association of metal ions or colorless polyphenolics (cofactors) to anthocyanins under acidic conditions. Copigment complexes also serve to enhance color and stability characteristics of anthocyanins in low acid conditions where anthocyanins are normally colorless. Several studies have suggested an increase in anthocyanin stability in the presence of cofactors (*12*−*14*), but directed studies relating copigmentation and AA stability are not reported in the literature. Shrinkhande and Francis (*15*) observed that flavonoid addition to anthocyanin−ascorbic−metal model systems prevented their degradation, results that were attributed to the antioxidant properties of flavonoids; other studies have also linked anthocyanin stability to the presence of flavonols (*16*, *17*). The chemical stability conferred by copigmented anthocyanins in the presence of AA is unknown and was selected for this study due to its potential to serve as a nutrient stabilizing agent during food processing and subsequent storage.

The present study evaluated the storage stability of red grape anthocyanins (*Vitis vinifera*) in the presence of AA and water soluble polyphenolic cofactors isolated from rosemary (*Rosmarinus officinalis*). Following thermal pasteurization of model juice systems, phytochemical and quality characteristics were monitored over time to assess the pigment stabilizing effects of rosemary cofactors (REs) in the presence of AA.

* To whom correspondence should be addressed. Tel: +52-818-358-20-00 ext. 4817. Fax: +52-818-328-4322. E-mail: chbrenes@itesm.mx.
† ITESM-Campus Monterrey.
‡ University of Florida.

10.1021/jf049857w CCC: $30.25
Published on Web 12/13/2004

MATERIALS AND METHODS

Materials and Processing. Red grape anthocyanins (Warner Jenkinson, St. Louis, MO) and a concentrated water soluble rosemary extract (Color Enhance-R, 3.5% rosemarinic acid; RFI Ingredients, Blauvelt, NY) were individually dissolved in 0.1 M citric acid buffer (pH 3.5) and further purified using activated C_{18} Sep-Pak Vac 20 cm^3 mini-columns (Waters Corporation, Milford, MA). Residual sugars and organic acids were removed with water (0.01% HCl), and polyphenolic compounds were recovered with acidified methanol (0.01% HCl) (*18*). Methanol was removed from the polyphenolic fraction using vacuum evaporation at <40 °C, and the resulting isolates were redissolved in a known volume of citric buffer.

In vitro model systems simulating a fruit juice were prepared using the purified red grape anthocyanins, high fructose corn syrup (10° Brix), and citric acid buffer adjusted to give a final absorbance value of 1.5 at 520 nm. The stock solution was partitioned into smaller quantities for application of treatment combinations. The purified rosemary extract (2.5 g/L gallic acid equivalents) was added to contain final concentrations in the juice models of 0, 0.2, and 0.4% v/v. Each copigment level was divided into two subfractions, one fortified with L-ascorbic acid (L-AA) (450 mg/L) and the other fortified with an equal volume of citric acid buffer as a nonfortified control. All treatment combinations were handled identically throughout the study and were adjusted to pH 3.5 when needed. Treatments were sealed in 20 mL screw-cap glass vials, pasteurized at 85 °C for 30 min in a hot water bath, and stored in the dark at 25 and 35 °C for 30 days. Unheated samples of each treatment were also collected for evaluation and were stored frozen (−20 °C) until analysis.

Phytochemical Analyses. Physicochemical changes in model systems following the addition of REs and AA were evaluated as a function of thermal pasteurization and storage conditions. Models systems were analyzed for color characteristics based on spectral properties of anthocyanins. Changes in color intensity were determined by monitoring shifts in maximum absorbance (% hyperchromic shifts) and wavelength (% bathochromic shifts). The percentage of monomeric/polymeric anthocyanins was determined based on color retention in the presence of potassium metabisulfite (*1*), while color density was determined by spectral measurements at 420, 520, and 700 nm according to Rodriguez-Saona (*1*). Additionally, instrumental CIE Color characteristics including lightness (L^*), chroma value, and hue angle were measured using a Minolta Chroma Meter CR-300 Series (Minolta Co., Ltd., Osaka, Japan). The anthocyanin content was determined on appropriately diluted samples by the pH differential spectrophotometric method of Wrolstad (*19*). Total soluble phenolics were measured using the Folin−Ciocalteu assay and were quantified as gallic acid equivalents (*20*). Total (TAA) and L-AA were quantified by reverse phase high-performance liquid chromatography (HPLC) using modified chromatographic conditions described by Gökmen et al. (*21*). Separation was performed on a Waters Nova-Pak C_{18} column (3.9 mm × 150 mm), using KH_2PO_4 (0.2 M, pH 2.4) as the mobile phase at a flow rate of 0.5 mL/min with UV detection at 254 nm. Prior to AA analysis, all samples were passed thorough preconditioned Waters C_{18} Sep-Pak cartridges (Waters), to remove neutral polyphenolics. The cartridges were preconditioned by washing with 4 mL of methanol followed by 10 mL of Nanopure water. The samples (4 mL) were bound to the cartridges, the first 2 mL were discarded, and the next 2 mL were collected and subsequently divided in two fractions for analysis of L-AA and TAA. L-AA samples were immediately filtered through a 0.45 μm PTFE filter (Millipore Co., Billerica, MA) and analyzed by HPLC. For TAA, dithiothreitol (8 mM) was added as a reducing agent and the samples were kept in the dark for 120 min to convert any dehydroascorbic acid (DHA) to L-AA. After complete conversion, the samples were filtered through a 0.45 μm PTFE filter (Millipore Co.) and analyzed for TAA by HPLC. The content of DHA in the sample was calculated by subtracting the L-AA content from TAA.

Statistical Analysis. The experiment was designed as a 3 × 2 × 8 [full factorial that included three RE levels (0, 0.2, and 0.4% v/v), evaluated in the absence or presence of AA (0, 450 mg/L), and sampled at eight storage times]. The phytochemical stability of model systems was monitored during normal (25 °C) and accelerated (35 °C) storage conditions. Data represent the mean of three replicates. Regression analyses, Pearson correlation coefficients, and analysis of variance were conducted using JMP software Version 5 (*22*), with mean separation performed by the least significant difference (LSD) test ($P < 0.05$).

Table 1. Effect of REs (0, 0.2, and 0.4% v/v) and AA Fortification (450 mg/L) on the Spectroscopic Properties of a Model Grape Juice System Following Thermal Pasteurization

	RE (% v/v)	no AA		added AA	
		unprocessed	pasteurized	unprocessed	pasteurized
hyperchromic shift (%)	0		0.01 b[a]	0.01 b	0.01 b
	0.2	151.7 b	145.0 b	131.7 b*[b]	113.3 b*
	0.4	178.3 a	163.3 a	161.7 a*	141.7 a*
bathochromic shift (nm)	0		0.6 c	1.8 c*	1.50 c
	0.2	16.8 b	16.0 b	17.2 b	14.7 b*
	0.4	23.0 a	19.8 a	23.0 a	20.0 a
color density	0	1.83 c	1.65 c	1.60 c*	1.47 c
	0.2	2.47 b	2.44 b	2.18 b*	2.10 b*
	0.4	2.99 a	2.97 a	2.70 a*	2.57 a*
lightness (L^*)	0	29.8 a	29.4 a	30.3 a	31.4 a
	0.2	24.2 b	24.3 b	25.7 b	25.5 b
	0.4	22.8 b	22.7 b	23.4 b	23.7 b
chroma value	0	38.0 a	37.7 a	39.3 a	38.9 a
	0.2	31.3 b	32.2 b	34.7 b	35.0 b
	0.4	28.2 b	28.2 c	31.8 b	31.7 b
hue angle	0	20.3 a	19.7 a	18.0 a	18.0 a
	0.2	13.2 b	13.7 b	13.0 b	13.7 b
	0.4	12.8 b	12.6 b	12.8 b	13.6 b

[a] Values with different letters within columns of each treatment and response attribute are significantly different (LSD test, $P < 0.05$) and indicate the effect of RE addition. [b] Treatments with an asterisk (*) for each RE level indicate a significant effect (LSD test, $P < 0.05$) due to AA addition when compared to the same treatment without AA.

Table 2. Effect of REs (0, 0.2, and 0.4% v/v) and AA Fortification (450 mg/L) on the Physicochemical Characteristics of a Model Grape Juice System Following Thermal Pasteurization

	RE (% v/v)	no AA		added AA	
		unprocessed	pasteurized	unprocessed	pasteurized
total anthocyanins[c] (mg/L)	0	835.9 a[a]	732.9 a	833.1 a	759.8 a*[b]
	0.2	722.8 b	688.2 b	726.4 b	678.3 b
	0.4	718.0 c	660.5 c	716.9 b	663.7 b
total AA (mg/L)	0			448.7 b	439.4 a
	0.2			478.2 a	445.1 a
	0.4			476.1 a	432.5 a
L-AA (mg/L)	0			417.4 b	386.8 a
	0.2			467.4 a	382.8 a
	0.4			462.2 a	375.3 a
monomeric anthocyanins (%)	0	74.7 ab	76.2 a	73.9 b	75.4 b*
	0.2	75.9 a	77.5 a	75.4 ab	76.9 a
	0.4	73.8 b	75.3 a	76.2 a*	77.8 a*
total soluble phenolics[d] (mg/L)	0	74.7 c	74.3 c	99.8 b*	107.8 b*
	0.2	120.5 b	122.5 b	150.8 a*	152.9 a*
	0.4	175.1 a	163.9 a	162.5 a	212.0 a*

[a] Values with different letters within columns of each treatment and response attribute are significantly different (LSD test, $P < 0.05$) and indicate the effect of RE addition. [b] Treatments with an asterisk (*) for each RE level indicate a significant effect (LSD test, $P < 0.05$) due to AA addition when compared to the same treatment without AA. [c] Anthocyanin content expressed as malvidin 3-glucoside equivalents. [d] Expressed as gallic acid equivalents.

RESULTS AND DISCUSSION

Effects of Copigmentation and AA Fortification. This first discussion section is based on the results from statistical analyses of data collected prior to the storage period and basically includes the main effects of rosemary extract addition and also discusses the effect of AA addition (**Tables 1** and **2**). Additional unprocessed samples were also collected for all treatments;

therefore, the effect of the thermal processing by itself on the different response variables is also discussed in this section.

Bathochromic and hyperchromic shifts in spectral properties of anthocyanins are commonly used as indicators of color augmentation by cofactors (*12*). Color changes were perceived visually in model systems, and as expected, hyperchromic (up to 178%) and bathochromic (up to 23 nm) shifts were correlated with increments in rosemary extract concentration ($r = 0.97$ and $r = 0.96$, respectively; **Table 1**). Both pasteurization and AA fortification led to a slight decrease in maximum absorbance values (ca. 0.2 absorbance units) and in bathochromic shifts (<2 nm). Instrumental color attributes (lightness, chroma values, and hue angles) were modified following rosemary extract addition but were not altered by pasteurization or AA fortification (**Table 1**). Copigment addition caused a decline in lightness values, possibly due to the actual contribution of yellow-brownish color of the phenolic extracts. Similarly, the addition of REs decreased chroma values indicating a slight decrease in the brightness of the systems. However, hue angle was the instrumental color parameter that presented the greatest decline and is also the colorimetric parameter that best describes the effect of copigmentation on visual perception. Copigmented model systems showed a decrease in hue angles (from 20.3 to 12.8) toward a more intense red color, a desirable trait that was statistically significant among treatments. Results for color density (**Table 1**), a spectral measurement of the absolute color of an anthocyanin solution, decreased slightly (<0.2) due to AA fortification and increased significantly due to copigment addition (36 and 66%, with correspondent increments of 0.2% v/v in cofactor concentration).

The initial anthocyanin content (836 mg/L; **Table 2**) of the juice model was decreased by 8% following the addition of REs. Such an effect is not likely attributed to anthocyanin degradation but rather due to the effect of copigmentation on the spectrophotometric method based on pH shifts. In this method, monomeric anthocyanins are readily altered at different pH values; however, the copigmented anthocyanin complex appears to remain colored since this association has been reported to be less sensitive to changes in pH (*12*). Therefore, by this assay, the total anthocyanin content of copigmented systems appears to be lower when compared to the controls. Additionally, and to account for the effect of bathochromic shifts that occurred following copigmentation, absorbance measurements in this total anthocyanin method were taken at the maximum wavelength of each system. The addition of AA did not cause an initial degradation of anthocyanins, but following pasteurization, a 12% loss in anthocyanins was observed, an effect that was reduced to <5% by the addition of REs. A similar color protection given by copigmentation during thermal processing was observed by Malien-Aubert et al. (*23*), but their systems did not contain AA. These authors found that the irreversible conversion to chalcones is a key step in the overall mechanism of anthocyanin degradation at high temperatures and suggested that copigment complexes may be decreasing anthocyanin degradation by lowering the molar ratio between colorless chalcones and colored anthocyanins via a selective complexation (*14, 23*).

An initial protective effect against TAA and L-AA degradation was also observed following the addition of REs; however, concentrations equilibrated and were similar to the control immediately after pasteurization, likely due to the significant losses ($p < 0.05$) observed for both forms of the vitamin after thermal processing (**Table 2**).

A small amount of anthocyanin polymerization occurred initially following the addition of rosemary extracts and AA, as indicated by a slight decrease (<2%) in the percent monomeric anthocyanins (**Table 2**). According to previous studies, the addition of external phenolic compounds may result in polymerization from condensation of monomeric anthocyanins or their complexation with phenolics. These polymers are resistant to color bleaching in the presence of bisulfite and result in lower values for % monomeric anthocyanins (*12*). As expected, the addition of REs and AA increased the total phenolic content in the model systems, since both contain appreciable metal reducing capacity in the Folin−Ciocalteu assay (**Table 2**). A 0.2% v/v increase in the content of REs caused a 50 mg/L linear increment of gallic acid equivalents on average. Similarly, AA fortification increased total phenolics by 31 mg/L gallic acid equivalents on average over the nonfortified control.

AA Degradation during Storage. The following sections will discuss the results that took place during the storage period. This particular section focuses on the trends observed for AA and its bioactive forms of vitamin C, L-AA (reduced), and DHA (oxidized). Results of this study suggest that AA degradation in the presence of anthocyanins takes place in oxidative steps that progress via the conversion of L-AA into DHA and subsequently to other condensation byproducts (*10*). The benefits of anthocyanin copigmentation with rosemary polyphenolics to delay AA degradation during normal (25 °C) and accelerated (35 °C) storage conditions are a novel finding in the context of commercial applications in food systems.

Nonsignificant differences in L-AA and TAA content were found during the first 2 days of storage at 25 °C between controls and treatments containing REs at the 0.2% v/v level (**Figure 1A**,**B**). However, at 2 days of storage, treatments containing REs at 0.4% v/v level had higher amounts of L-AA and TAA when compared to the control, whereas differentiation was only apparent after 7 days for the lower concentration (0.2% v/v). The L-AA content at 11 days of storage was positively correlated to cofactor concentrations, and systems containing rosemary copigments at 0.2 and 0.4% v/v retained 50 and 130 mg/L more L-AA, respectively, when compared to the control. For the control treatment, neither DHA (**Figure 1C**) nor TAA (**Figure 1A**) was detectable after 15 days of storage; yet, significant amounts of TAA (60−75 mg/L) still remained in both copigmented treatments due to residual DHA. Again, overall results from storage at 25 °C indicated that the addition of REs at 0.4% v/v kept AA in its reduced state (L-AA) preventing its conversion into DHA. In contrast, juices containing the lower concentration of REs (0.2%) presented similar L-AA degradation patterns than the control but extract addition appeared to slow the further breakdown of DHA after 8 days of storage thus resulting in higher vitamin retention (higher TAA).

For model systems stored at 35 °C, no differences in L-AA and TAA contents were found until day 5 (**Figure 2A**,**B**), after which L-AA decreased linearly during storage. After 11 days of storage, models containing REs at 0.2 and 0.4% v/v retained slightly higher concentrations of L-AA than controls (35 and 50 mg/L, respectively). However, treatment differences relative to the control were more noticeable for TAA after 7 days of storage (>100 mg/L) and were again attributable to residual DHA content (**Figure 2C**). Results showed that the addition of REs influenced the overall vitamin retention by delaying the further degradation of DHA, even under the accelerated storage conditions. Moreover, copigment addition can also benefit color retention by retarding the degradation of L-AA and DHA into

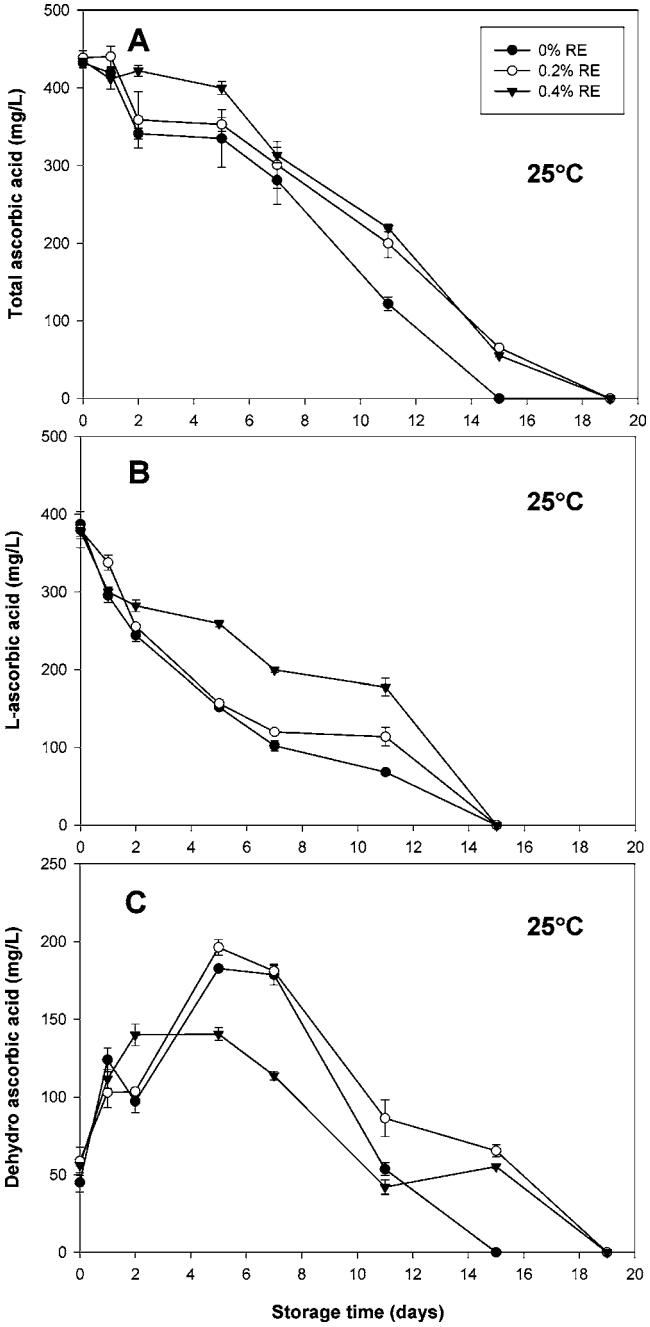

Figure 1. Total ascorbic (**A**), L-ascorbic (**B**), and dehydroascorbic (**C**) acid contents of a model grape juice system stored at 25 °C as influenced by the presence of REs (0, 0.2, and 0.4% v/v). Bars represent standard errors of the mean ($n = 3$).

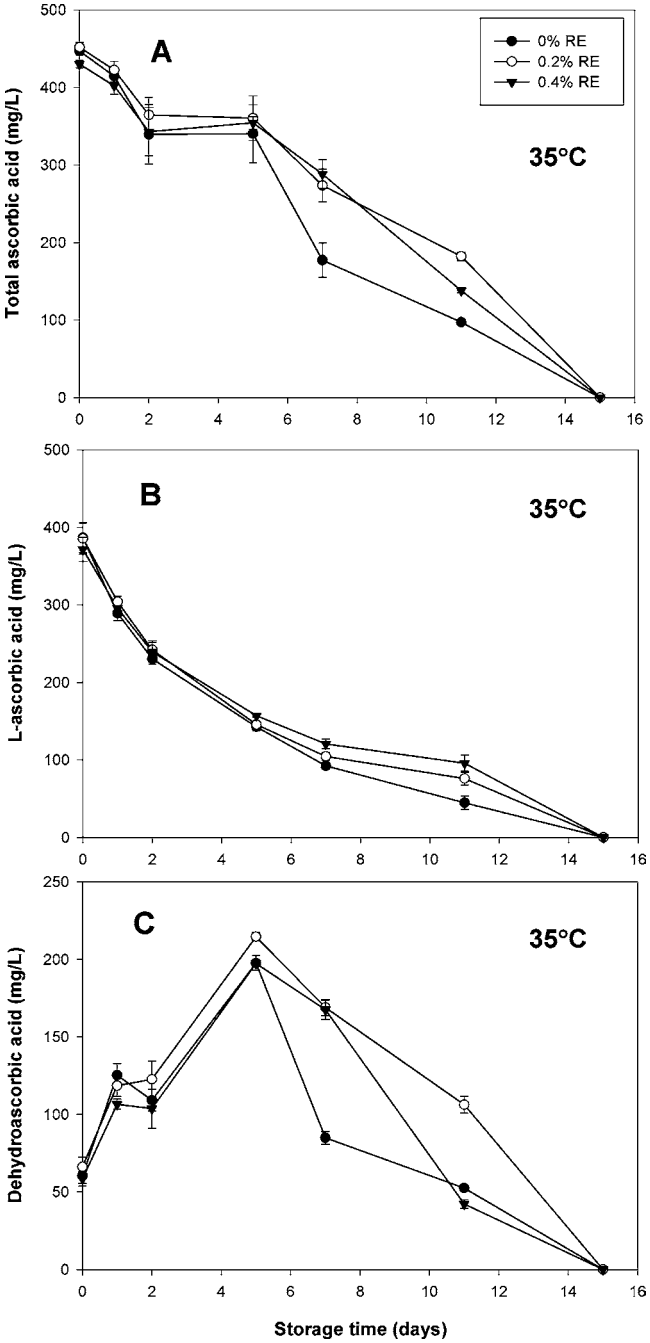

Figure 2. Total ascorbic (**A**), L-ascorbic (**B**), and dehydroascorbic (**C**) acid contents of a model grape juice system stored at 35 °C as influenced by the presence of REs (0, 0.2, and 0.4% v/v). Bars represent standard errors of the mean ($n = 3$).

products such as 2,3-diketogulonic acid and various aldehydes that have been shown to accelerate anthocyanin degradation (*24*).

Anthocyanin Degradation during Storage of Juice Model Systems. For all models under investigation, the storage stability of anthocyanins was considerably higher than that of AA. In the absence of AA, the addition of copigments did not present a stabilizing effect on grape anthocyanins stored at 25 °C (**Figure 3A**) with an average anthocyanin loss of 67% after 30 days of storage. For treatments containing AA, anthocyanin losses were similar to nonfortified treatments during the first 11 days of storage after which the presence of AA significantly accelerated anthocyanin destruction. This was presumably due to formation of byproducts from AA and carbohydrate degradation, such as furfurals and other aldehydes, that accelerated pigment degradation and negatively impacted juice quality as previously reported (*24*, *25*). In their previous studies, the mechanism of degradation was proposed as a condensation reaction between furfural and hydroxyl groups of the anthocyanin B ring yielding brown, polymerized pigments. In this study, the presence of REs alleviated anthocyanin degradation, as copigmented treatments fortified with AA experienced losses of 82% that were only 15% higher than nonascorbic containing counterparts. It is possible that rosemary polyphenolic cofactors likely exerted a protective effect against anthocyanin degradation in the presence of AA degradation products such as aldehydes, as cofactors compete with anthocyanins and preferentially react in the proposed condensation reactions (*24*, *25*).

Treatments without AA and stored at 35 °C (**Figure 3B**) maintained higher anthocyanin contents (>100 mg/L) after 15

Table 3. Effect of REs (0, 0.2, and 0.4% v/v) on Kinetic Parameters of TAA and L-AA Degradation in Juice Model Systems Stored at 25 and 35 °C

storage temp	RE (%v/v)	TAA					L-AA				
		β_0	β_1[a]	R^2	$t_{1/2}$[b]	Q_{10}[c]	β_0	β_1	R^2	$t_{1/2}$	Q_{10}
25 °C	0	0.082	–0.104	0.87	7 c[d]	1.21	–0.106	–0.158	0.9	4 c	1.31
	0.2	0.032	–0.068	0.93	10 b	1.26	–0.112	–0.119	0.89	6 b	1.15
	0.4	0.064	–0.059	0.89	12 a	1.95	–0.109	–0.064	0.92	11 a	1.53
35 °C	0	0.077	–0.137	0.92	5 c		–0.071	–0.191	0.99	4 c	
	0.2	0.019	–0.078	0.94	9 a		–0.119	–0.150	0.97	5 b	
	0.4	0.061	–0.092	0.86	8 b		–0.132	–0.124	0.95	6 a	

[a] Indicates the linear regression intercepts (β_0) and degradation rates (slope, β_1) of TAA and L-AA (days^{-1}). [b] $t_{1/2}$ indicates the half-life (days) of initial AA content. [c] Indicates temperature-dependent quotients of AA degradation as affected by increments in storage temperature from 25 to 35 °C. [d] Values with similar letters within columns of each storage temperature are not significantly different (LSD test, $P < 0.05$).

Table 4. Effect of REs (0, 0.2, and 0.4% v/v) and AA (450 mg/L) Fortification on Kinetic Parameters of Anthocyanin Degradation in Juice Model Systems Stored at 25 and 35 °C

storage temp	RE (%v/v)	no AA					added AA				
		β_0	β_1[a]	R^2	$t_{1/2}$[b]	Q_{10}[c]	β_0	β_1	R^2	$t_{1/2}$	Q_{10}
25 °C	0	–0.0809	–0.035	0.97	20 c[d]	1.67	0.1573	–0.101	0.96	7 c	1.44
	0.2	–0.0038	–0.032	0.98	22 b	1.13	0.0812	–0.078	0.87	9 b	1.72
	0.4	0.0074	–0.031	0.98	23 a	1.15	0.0670	–0.062	0.98	11 a	2.22
35 °C	0	–0.1714	–0.058	0.97	12 c		0.0894	–0.145	0.94	5 a	
	0.2	–0.1287	–0.037	0.93	19 b		0.1333	–0.134	0.95	5 a	
	0.4	–0.0979	–0.035	0.96	20 a		0.1509	–0.138	0.95	5 a	

[a] Indicates the linear regression intercept (β_0) and degradation rate (slope, β_1) of anthocyanins (days^{-1}). [b] $t_{1/2}$ indicates the half-life (days) of initial anthocyanin content. [c] Indicates temperature-dependent quotients of anthocyanin degradation as affected by increments in storage temperature from 25 to 35 °C. [d] Values with similar letters within columns of each storage temperature and presence or absence of AA are not significantly different (LSD test, $P < 0.05$).

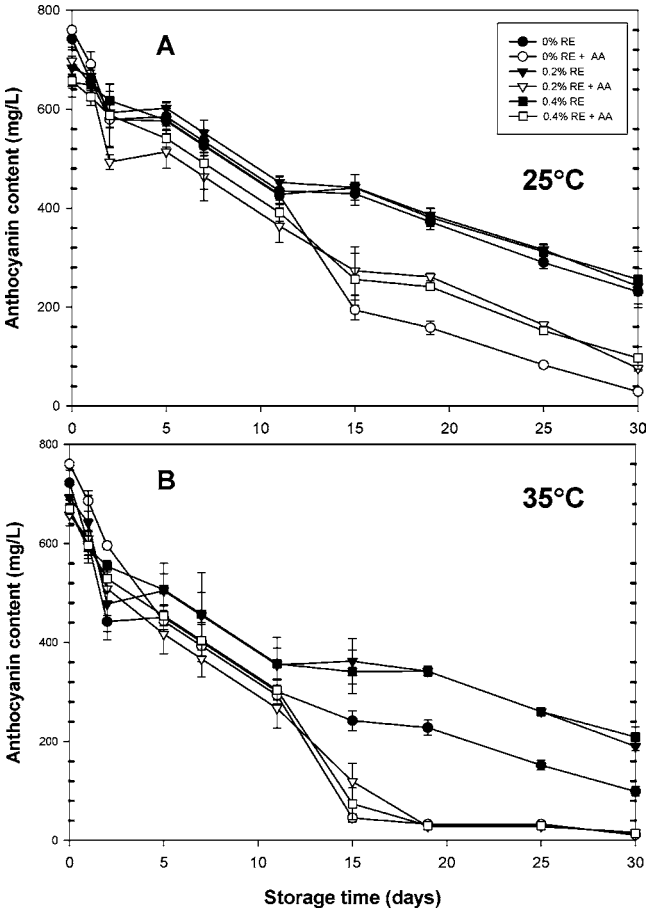

Figure 3. Anthocyanin content in a model grape juice system during normal (25 °C; **A**) and accelerated (35 °C; **B**) storage as influenced by REs (0, 0.2, and 0.4% v/v) and AA (450 mg/L). Bars represent standard errors of the mean ($n = 3$).

days of storage in the presence of REs. The protective effect exerted by anthocyanin copigment complexes was also evident in the presence of AA; however, it was appreciably reduced at this temperature due to the rate, sequence, and multiplicity of degradative reactions simultaneously occurring to anthocyanins, AA, and polyphenolic cofactors.

Kinetics of AA and Anthocyanin Degradation. Regression analysis was used to determine the adequacy of the model describing kinetics of AA destruction and confirmed that degradation rates followed first-order kinetics ($P < 0.05$), in agreement with previous reports (*9*). First-order rate constants (β_1) that describe the destruction of L-AA and TAA and their half-lives ($t_{1/2}$), which indicate the time needed for 50% degradation, were calculated using the following equations (*26*) and are shown in **Tables 3** and **4**: $\ln(A_t/A_o) = -\beta_1 t$; $t_{1/2} = (\ln 0.5)/\beta_1$, where A_o was the initial AA or anthocyanin concentration and A_t was the phytochemical concentration at a given storage time (t). Comparison of the kinetic parameters among treatments indicated that degradation rates for both TAA and L-AA slowed as rosemary extract concentrations increased. The half-life values ($t_{1/2}$) for TAA were extended from 7 days for the control to 10 and 12 days for 0.2 and 0.4% v/v RE levels, respectively, when stored at 25 °C, and increased from 5 to 8 days for both rosemary phenolic concentrations at 35 °C (**Table 3**). Similar trends were observed for L-AA, where REs extended L-AA half-life in a concentration-dependent manner that was statistically significant.

Kinetic analysis indicated that anthocyanin degradation also fit first-order reaction kinetics ($P < 0.05$) in agreement with Garzon and Wrolstad (*9*). As expected, the presence of AA in the systems resulted in higher anthocyanin degradation rates (β_1; **Table 4**) and confirmed the destructive interaction between both compounds when present together in a food system. The beneficial effect of copigmentation on anthocyanin stability was evident in the extension of pigment $t_{1/2}$ values from 20 days for the control to 22 and 23 days in the presence of 0.2 and 0.4% of REs, respectively, at 25 °C. Likewise, in the presence of AA, $t_{1/2}$ was extended from 7 days in the control to 9 and 11 days (**Table 4**). A similar trend in the extension of anthocyanin shelf life caused by copigmentation was observed at 35 °C for nonvitamin containing samples; however, no protective effect

due to copigmentation was noted in the presence of AA ($t_{1/2}$ = 5 days).

The effect of storage temperature on AA retention was also determined by calculating temperature quotients ($Q_{10} = \beta_{1at25°C}/\beta_{1at35°C}$) between 25 and 35 °C (**Table 3**). A 10 °C increase in storage temperature resulted in 1.2 times faster degradation of TAA for the control and the copigmented treatment at 0.2%. Degradation was nearly doubled for models containing 0.4% rosemary extract ($Q_{10} = 1.96$), possibly reflecting polyphenolic oxidation and quinone formation, an additional destructive factor at higher storage temperatures, caused by the externally added phenolic compounds present in the systems.

Temperature quotients (Q_{10}) were also used to assess the effect of storage temperature dependence on anthocyanin degradation. An increase in storage temperature from 25 to 35 °C significantly increased anthocyanin degradation (1.7-fold) for the control treatment without AA, yet only slightly increased anthocyanin degradation in both copigmented models (average = 1.14) as shown in **Table 4**, also indicating a higher stability of these systems. The opposite effect was observed in the presence of AA where anthocyanin and AA degradation were accelerated at higher temperatures, again possibly influenced by a higher reactivity of the additional rosemary phenolics present in model systems.

Comparison of the Degradation Kinetics of AA and Anthocyanins. So far, the degradation kinetics of AA and anthocyanins alone have been previously discussed; however, their mutual degradation in food systems is well-known and therefore is the focus of this discussion. For this section only, the systems containing both anthocyanins and AA are being discussed since they are the ones involved in the relationship under study. For these model juices, TAA was the parameter that presented the best correlation with anthocyanin losses at both 25 and 35 °C (r = 0.97 and 0.96, respectively), thus indicating that further degradation products from DHA are important contributors to anthocyanin destruction. The oxidation of L-AA in to DHA is only partially contributing to the anthocyanin losses, as it can be appreciated by the lower $t_{1/2}$ values for L-AA at 25 °C (**Table 3**) when compared to the higher $t_{1/2}$ values for the anthocyanins in the fortified systems (**Table 4**). Anthocyanin $t_{1/2}$ values are closer to those of TAA, possibly indicating that when the reaction proceeded further (DHA declined) the most intense pigment losses were observed. For models containing REs, kinetic analysis confirmed that the degradation rates (β_1) for both L-AA and TAA were decreased by copigment addition. Additionally, anthocyanin degradation started its most rapid decline about 10 days into storage when only 50% of the TAA was remaining, suggesting that the prevention of the degradation of both L-AA and DHA is a key step in order to further slow the degradation of anthocyanins.

The observed stabilization of anthocyanin−AA containing systems through copigmentation is an important observation that will require further elucidation to understand the mechanisms involved. It is possible that copigmentation may result in a free radical stabilization effect or result in a steric hindrance of condensation reactions involving *o*-quinones or aldehydes (*14*, *24*, *25*). Additionally, hydrophobic complexation reactions between anthocyanins and cofactors may effectively protect anthocyanins against nucleophilic water attack at position 2 of the pyrilium nucleus, thus displacing the equilibra toward the flavilium form (colored) rather than that of the less-colored hemiketal or chalcone forms (*1*, *10*, *27*).

Anthocyanin Polymerization and Changes in Color Indices during Storage. This last section focuses on the discussion

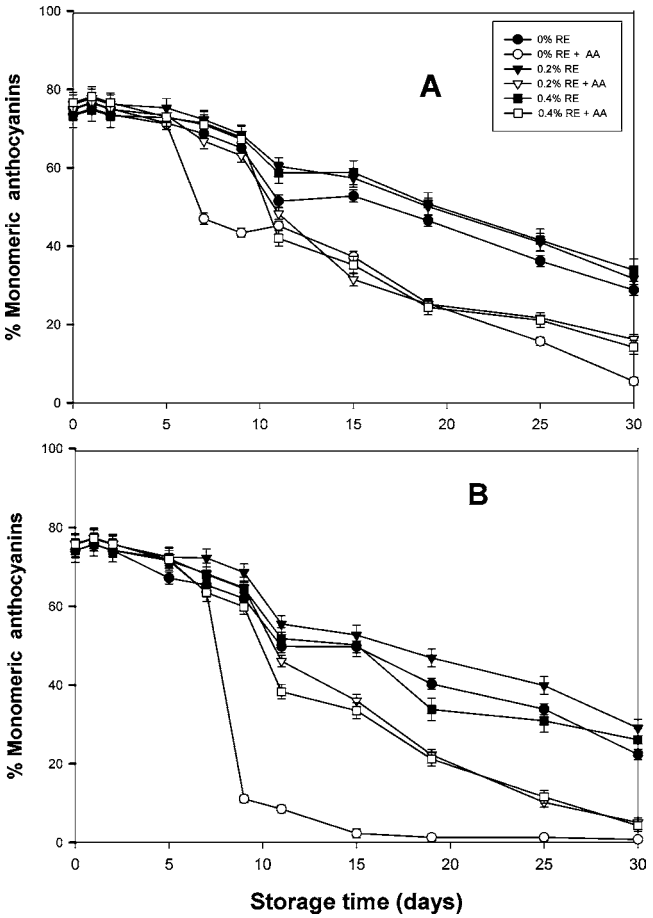

Figure 4. Percent monomeric anthocyanins as influenced by the presence of REs (0, 0.2, and 0.4% v/v) and AA fortification (450 mg/L) during storage of model juice systems under normal (25 °C; **A**) and accelerated (35 °C; **B**) conditions. Bars represent standard errors of the mean (n = 3).

of the variation in color indices during the storage period. The addition of REs did not reduce anthocyanin polymerization reactions in the absence of AA for systems stored at 25 °C as indicated by the similar decline in percent monomeric forms from 75 to 25% during storage (**Figure 4A**). However, when models had AA present, copigmentation prevented anthocyanin polymerization by more than 10% with respect to the control, which contained mostly polymerized forms (95%). In the presence of AA and during the storage of models at 35 °C, anthocyanin polymerization was significantly reduced following the addition of REs and resulted in ∼50% greater retention of monomeric anthocyanins when compared to the control (**Figure 4B**). Again, the later effect likely occurred due to a decrease in the production of secondary byproducts from AA degradation or interaction of these products with REs that served to slow degradative reactions.

Significant changes ($P < 0.05$) in CIE color parameters were observed in all treatments during storage and followed similar trends at 25 (**Figure 5**) and 35 °C (data not shown). The negative effect on color caused by AA fortification was readily distinguishable and resulted in color fading (increased L^* value) toward an orange color (increased hue angles). However, copigmentation served to mask these detrimental changes, since juices containing REs had lower hue angles that were visually perceived as a more intense red color. Despite AA fortification, the absolute color of each model system (measured through hue angles) generally increased from red toward orange and yellow throughout storage for all treatments. Results were indicative

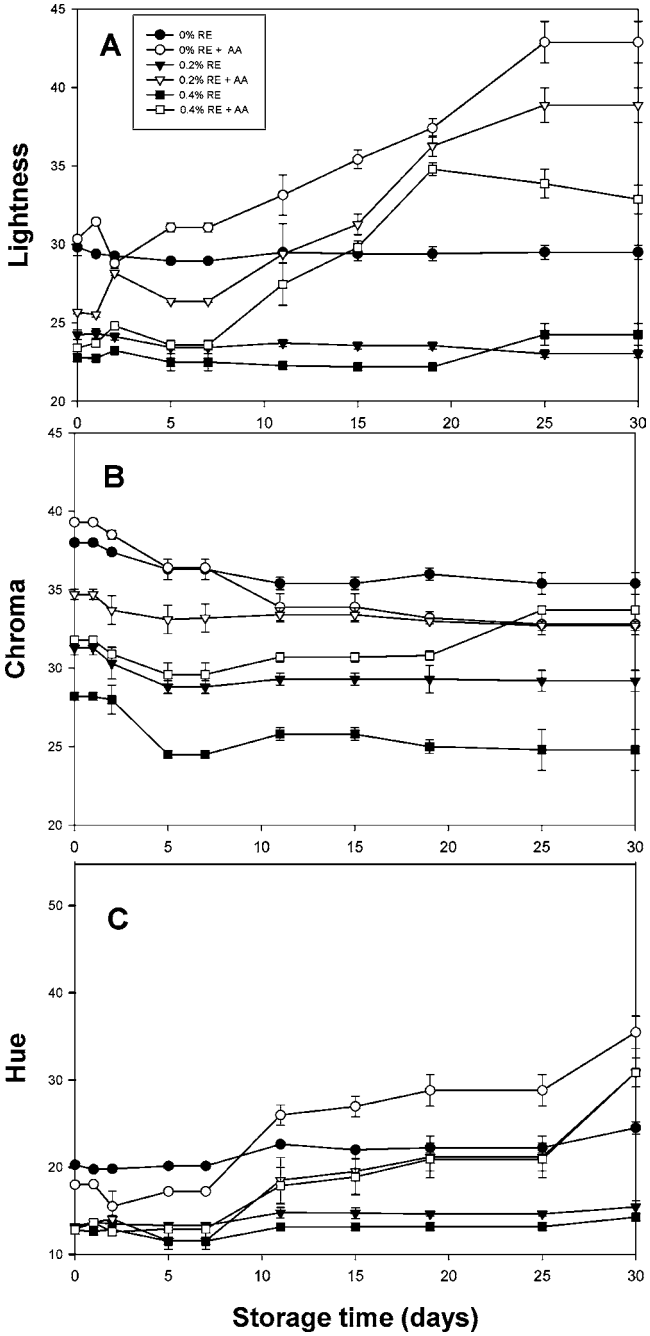

Figure 5. CIE lightness (**A**), chroma (**B**), and hue angles (**C**) color parameters during storage of model juice systems under normal conditions (25 °C) as influenced by the presence of REs (0, 0.2, and 0.4% v/v) and AA (450 mg/L). Bars represent standard errors of the mean ($n = 3$).

of product browning and the formation of quinoidal bases and chalcones during storage. Changes in hue angle during storage were directly correlated to anthocyanin degradation ($r = 0.80$ for 25 °C and $r = 0.84$ for 35 °C). The addition of REs served to alleviate this color degradation (independently of AA content) and overall maintained a more red color than their respective controls. After 19 days of storage, models containing equivalent rosemary concentrations at either 25 or 35 °C resulted in similar hue angles and remained constant throughout the remainder of the study.

During storage, similar color density losses (~27%) were observed for all model systems for both temperatures (data not shown). Rates of color density loss were not significantly affected by either presence of AA or REs ($P > 0.05$) but were significantly increased by a higher storage temperature (26%). A slower rate of color density loss was observed in comparison with that of anthocyanins, which is in agreement with previous studies (*9*, *10*, *28*).

This study revealed that the addition of polyphenolic-based cofactors extracted from rosemary reduces the mutual destruction of anthocyanins and L-AA by delaying its conversion to DHA, which is a precursor to additional compounds believed to result in the destruction of anthocyanins. Copigmentation also appreciably augmented visual color and masked the detrimental changes that took place during storage as a result of phytonutrient degradation. In addition to preventing quality and nutritional losses, copigmentation improved oxidative and thermal stability of anthocyanins, which can benefit the economic value of many products that contain them through shelf life extension. Other than the present study, no information was available on phytonutrient retention being enhanced in the presence of copigmented anthocyanins. In the models under study, copigmentation proved to be a complex balance between quality and nutrient retention and accelerated rates of oxidation, particularly at high storage temperatures. Because of that, a better understanding of the complex interactions between system components (anthocyanins, AA, and polyphenolics) is required in order to improve the performance of copigmentation in an actual beverage system.

ABBREVIATIONS USED

L-AA, L-ascorbic acid; DHA, dehydroascorbic acid; TAA, total ascorbic acid; RE, rosemary cofactor.

LITERATURE CITED

(1) Rodriguez-Saona, L. E.; Giusti, M. M.; Wrolstad, R. E. Color and pigment stability of red radish anthocyanins and red-fleshed potato anthocyanins in juice model systems. *J. Food Sci.* **1999**, *61*, 688−694.

(2) Miniati, E.; Damiani, P.; Mazza, G. Copigmentation and self-association of anthocyanins in food model systems. *Ital. J. Food Sci.* **1992**, *4*, 109−116.

(3) Meyer, A. S.; Yi, O.; Pearson, D. A.; Waterhouse, A. L.; Frankel, E. N. Inhibition of human low-density lipoprotein in relation to composition of phenolic antioxidants in grapes (*Vitis vinifera*). *J. Agric. Food Chem.* **1997**, *45*, 1638−1643.

(4) Frankel, E. N.; Waterhouse, A. L.; Teissedre, P. L. Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *J. Agric. Food Chem.* **1995**, *43*, 890−894.

(5) Skrede, G.; Wrolstad, R. E.; Durst, R. W. Changes in anthocyanins and polyphenolics during juice processing of highbush blueberries (*Vaccinium corymbosum* L.). *J. Food Sci.* **2000**, *65*, 357−364.

(6) Clifford, M. N. Review: Anthocyanins−Nature, occurrence and dietary burden. *J. Sci. Food Agric.* **2000**, *80*, 1063−1072.

(7) Jurd, L. Some advances in the chemistry of anthocyanins-type pigments. In *The Chemistry of Plant Pigments*; Academic Press: Chichester, NY, 1997; pp 123−142.

(8) Poei-Langston, M. S.; Wrolstad, R. E. Color degradation of ascorbic-anthocyanin-flavanol model system. *J. Food Sci.* **1981**, *46*, 1218−1222.

(9) Garzon, G. A.; Wrolstad, R. E. Comparison of the stability of pelargonidin-based anthocyanins in strawberry juice and concentrate. *J. Food Sci.* **2002**, *67*, 1288−1299.

(10) Garcia-Viguera, C.; Bridle, P. Influence of structure on color stability of anthocyanins and flavylium salts with ascorbic acid. *Food Chem.* **1999**, *64*, 21−26.

(11) Iacobucci, G. A.; Sweeny, J. G. The chemistry of anthocyanins and related flavylium salts. *Tetrahedron* **1983**, *23*, 1057−1064.

(12) Boulton, R. The copigmentation of anthocyanins and its role in the color of red wine: A critical review. *Am. J. Enol. Viticult*. **2001**, *52*, 67−86.
(13) Asen, S.; Steward, R. N.; Norris, K. H. Co-pigmentation of anthocyanins in plant tissue and its effect on color. *Phytochemistry* **1972**, *11*, 1139−1145.
(14) Markakis, P. Stability of anthocyanins in foods. In *Anthocyanins as Food Colors*; Markakis, P., Ed.; Academic Press: New York, NY, 1982; pp 163−180.
(15) Shrinkhande, A. J.; Francis, F. J. Effect of flavonols on ascorbic acid and anthocyanin stability in model systems. *J. Food Sci*. **1979**, *39*, 904−906.
(16) Clegg, M. K.; Morton, A. D. Phenolic compounds of black currant juice and their protective effect on ascorbic acid. *J. Food Technol*. **1968**, *3*, 227−230.
(17) Özkan, M.; Yemenicioglu, A.; Asefi, N.; Cemeroglu, B. Degradation kinetics of anthocyanins from sour cherry, pomegranate, and strawberry juices by hydrogen peroxide. *J. Food Sci.* **2002**, *67*, 525−529.
(18) Giusti, M. M.; Wrosltad, R. E. Characterization of red radish anthocyanins. *J. Agric. Food Chem.* **1996**, *61*, 322−326.
(19) Wrolstad, R. E. Color and pigment analysis in fruit products. *Oregon Agric. Exp. Stn. Corvallis.* **1976**, Bulletin 624.
(20) Talcott, S. T.; Howard, L. R.; Brenes, C. H. Antioxidant changes and sensory properties of carrot puree processed with and without periderm tissue. *J. Agric. Food Chem*. **2000**, *48*, 1315−1321.
(21) Gokmen, V.; Kahraman, N.; Demir, N.; Acar, J. Enzymatically validated liquid chromatographic method for the determination of ascorbic and dehydroascorbic acids in fruit and vegetables. *J. Chromatogr. A* **2000**, *881*, 309−316.
(22) SAS Institute, Inc. *JMP Software Version 5*; SAS Institute, Inc.: Cary, NC, 2002.
(23) Malien-Aubert, C.; Dangles, O.; Amiot, M. J. Color stability of commercial anthocyanin-based extracts in relation to the phenolic composition: Protective effects by intra- and intermolecular copigmentation. *J. Agric. Food Chem.* **2001**, *49*, 170−176.
(24) Es-Safi, N.; Cheynier, V. R.; Moutounet, M. Role of aldehydic derivatives in the condensation of phenolic compounds with emphasis on the sensorial properties of fruit-derived foods. *J. Agric. Food Chem.* **2002**, *50*, 5571−5585.
(25) Es-Safi, N.; Fulcrand, H.; Cheynier, V.; Moutounet, M. Studies on the acetaldehyde-induced condensation of (−)-epicatechin and malvidin 3-*O*-glucoside in a model solution system. *J. Agric. Food Chem.* **1999**, *47*, 2096−2102.
(26) Taoukis, P. S.; Labuza, T. P.; Saguy, I. S. Kinetics of food deterioration and shelf life prediction. In *Handbook of Food Engineering Practice*; Valentas, K. J., Rotstein, E., Singh, R. P., Eds.; CRC Press: New York, NY, 1997; pp 361−403.
(27) Mazza, G.; Brouillard, R. The mechanism of co-pigmentation of anthocyanins in aqueous solutions. *Phytochemistry* **1990**, *29*, 1097−1102.
(28) Marti, N.; Pérez-Vicente, A.; García-Viguera, C. Influence of storage temperature and ascorbic acid addition on pomegranate juice. *J. Sci. Food Agric.* **2002**, *82*, 217−22.

Received for review January 27, 2004. Revised manuscript received September 28, 2004. Accepted October 11, 2004. This research was possible thanks to the support of a grant from the U.S. Department of Agriculture Foreign Agricultural Service (SCRP, Grant Agreement 00092832) and by the International Foundation for Science (IFS), Stockholm, Sweden, Grant E/3174.

JF049857W