JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

Stability and Enhancement of Berry Juice Color

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Attractive color is one of the main sensory characteristics of fruit and berry products. Unfortunately, the color of red juices is unstable and easily susceptible to degradation, leading to a dull and weak juice color. This study was designed to investigate the color stability and copigmentation of four different berry juices enhanced by phenolic acids and commercial color enhancers. Phenolic acid enrichment improved and stabilized the color of the berry juices during storage. The commercial color enhancers immediately produced an intensive color to the juices, which, however, was not very stable. The color enhancement was intensive in strawberry and raspberry juices and effective in lingonberry and cranberry juices. Sinapic acid induced the strongest color in strawberry juice. Ferulic and sinapic acids improved raspberry juice color equally. Rosmarinic acid enhanced the color of lingonberry and cranberry juices the most. The addition of the simple cinnamic acids produced novel peaks to the end of the high-performance liquid chromatography chromatogram, indicating a formation of new compounds. It can be assumed that sinapic and ferulic acids formed new intramolecular copigmentation compounds with berry anthocyanins whereas rosmarinic acid stabilized anthocyanins intermolecularly.

KEYWORDS: Anthocyanins; phenolic acids; color enhancement; storage stability

INTRODUCTION

Anthocyanins are responsible for the brilliant red color and its different hues in many fruits and berries. Attractive color is one of the main sensory characteristics of fruit and berry products, and this important quality parameter strongly affects consumer behavior. Unfortunately, the color of red juices is unstable and easily susceptible to degradation leading to a dull, weak, and brownish juice color. The color stability of anthocyanins is influenced by pH, storage temperature, presence of enzymes, light, structure and concentration of the anthocyanins, and the presence of other berry compounds such as other flavonoids and phenolics (*1*).

The consumption of berry products has been world widely encouraged because of their possible health benefits (*2*, *3*). To ensure the lure of berry juices, their color needs to be stabilized and the shelf life needs to be prolonged by enhanced color. The use of berry's own natural color enhancers, i.e., other phenolics, ought to be looked into. In our previous study, it was clearly shown that phenolic acids enhance and stabilize anthocyanin color (*4*). However, copigmentation fortification by phenolic acids in order to improve berry juice color has been scarcely reported. Maccarone et al. (*5*) stabilized blood orange juice color with rutin and caffeic acid addition, and Talcott et al. (*6*) improved grape juice color by anthocyanin copigmentation with a rosemary extract. Other means of stabilizing berry juice color have been reported previously to a lesser extent. Addition of sulfur dioxide slowed anthocyanin degradation in strawberry juice and purée (*7*), but then, ascorbic acid addition resulted in the loss of pigment stability (*8*). Metallic salts have also been found to stabilize strawberry purée color (*9*).

This study was designed to investigate the color stability of four different berry juices and the enhancement of their anthocyanin color by phenolic acids and commercial colorants. It was also of interest to examine the effect of different preparation methods of the juice on color stability (**Chart 1**).

MATERIALS AND METHODS

Sample Preparation. Frozen lingonberries (*Vaccinium vitis-idaea* L.) and cranberries (*Vaccinium oxycoccus* L.) from the previous summer crop were thawed and crushed before centrifuging to gain juice from them. The juice was clarified with a 10-fold pile of GF/A fiberglass filters (Whatman), after which it was further filtered through a 0.8 μm membrane filter (Gelman). Strawberry (*Fragaria ananassa*) and raspberry (*Rubus ideaeus* L.) juices (Brämhults Ab, Sweden) were brought from local supermarket and processed as above. Strawberry and raspberry juices consisted of 35% berries, added sugar, and water. Lingonberry and cranberry juices were diluted with Milli-Q water to 35% juice.

The copigments ferulic acid, sinapic acid, and rosmarinic acid were purchased from Extrasynthese (Geney, France). They were added individually into the juice 10-fold to the anthocyanin amount. Grape skin extract (Enocyanin), containing 3% anthocyanins and 97% carbohydrates as carriers, mainly maltodextrine, and black carrot extract, containing 3% anthocyanins and 97% carbohydrates as carriers, mainly maltodextrine, were purchased from Sensient Food Colors GmbH (Geesthacht, Germany, formarly DrMarcus GmbH), and Color'Plus, a

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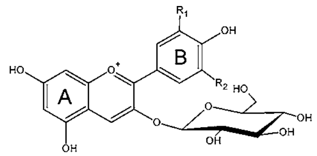
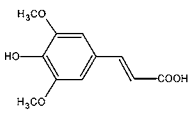
10.1021/jf035507i CCC: $27.50
Published on Web 04/16/2004

Chart 1. Structures of Anthocyanins and Phenolic Acids Used for Copigmentation Studies

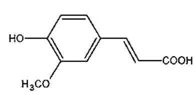


The most common anthocyanin monoglucosides

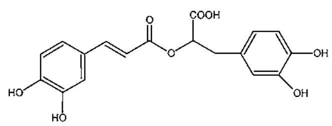
	R1	R2
Pelargonidin 3-glucoside	H	H
Cyanidin 3-glucoside	OH	H
Delphinidin 3-glucoside	OH	OH
Peonidin 3-glucoside	OCH_3	H
Petunidin 3-glucoside	OH	OCH_3
Malvidin 3-glucoside	OCH_3	OCH_3



Sinapic acid



Ferulic acid



Rosmarinic acid

nonanthocyanin rosmary extract, was purcahsed from Hurme Companies (Turku, Finland). These color enhancers were dissolved into strawberry and raspberry juices in the amount of 2 g/L of juice. The juices were then kept in an ultrasonic bath, lingonberry and cranberry for 10 min and strawberry and raspberry for 45 min, to ease the copigment dissolvement and also for sterilization of the two latter juices. The juices were kept at room temperature in daylight in sealed, but not vacuum-sealed, tubes to avoid evaporation for 103 days, during which their spectra and chromatic characteristics were recorded. The anthocyanin content was monitored by high-performance liquid chromatography (HPLC) in the beginning and the end of storage.

Compositional Analysis. In the beginning of the storage, the juice pH was verified using a Metler Delta 350 pH meter with pHC3359-9 combined electrode (Radiometer). The sugar content was measured with a Carl Zeiss 38091 refractometer. The total phenolic content was calculated according to the Folin–Ciocalteu method (1992) and expressed as gallic acid equivalents (GAE), milligrams per liter of juice. The ascorbic acid content was determined using an Enzymatic BioAnalysis kit (Boehringer Mannheim).

HPLC Analysis. The anthocyanin content was analyzed with a HPLC system (Waters, Milford, U.S.A.) consisting of a 2690 separation module, a PDA996 diode array detector, and a Millenium 2020C/S software data module. The analytical separation of anthocyanins was carried out on a Zorbax SB C18 column (150 mm × 4.6 mm, 5 μm; Agilent, U.S.A.) equipped with a C18 guard column. The temperature of the column oven was set to 40 °C. The mobile phase consisted of 10% formic acid (solvent A) and 100% CH_3CN (solvent B). The elution conditions were as follows: linear gradient from 5% B to 9% B, 0–5 min; to 11% B, 5–15 min; to 30% B, 15–20 min; to 95% B, 20–21 min; isocratic elution 95% B 21–24 min; linear gradient from 95% B to 5% B, 24–25 min; post-time 10 min before next injection. The flow rate was 1.0 mL/min, and the injection volumes were 30–100 μL. Detection wavelengths were 280 and 520 nm, and the spectra from 200 to 600 nm were recorded.

Colorimetric Measurements. The absorption spectra were recorded using a UV–visible spectrophotometer (Perkin-Elmer), scanning the visible range from 450 to 600 nm. The change in the maximum absorbance (A_{max}) at varying wavelengths (λ_{max}) presented the change in the color intensity and revealed a possible hyperchromic effect and bathochromic shift resulting from a copigmentation reaction.

CIELAB parameters were determined with Minolta Chroma Meter CR-210 colorimeter (Japan) using the illuminant D_{65} diffused illumination. The parameters gained were L^* for lightness, a^* for redness, and b^* for yellowness. These calculations of C^* for chroma, h for hue angle, and ΔE for total color change were made with the following equations:

$$C^* = [(a^*)^2 + (b^*)^2]^{1/2} \quad (1)$$

$$h = \arctan (b^*/a^*) \quad (2)$$

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (3)$$

Statistical Analysis. Statistical analysis of variance was conducted using Statgraphics plus, version 3.2. Significant ($P < 0.05$) differences between means of four (colorimetric measurements) or three (HPLC and juice composition measurements) replicates were identified using Tukey's procedure.

RESULTS

Enhancement of Juice Color. *Spectral Analyses.* Phenolic acid enrichment to four different berry juices improved the juice color during 103 days of storage. The enhancement of the color during storage was more intensive in strawberry and raspberry juices than in lingonberry and cranberry juices, measured as the hyperchromic effect of λ_{max} of the absorption spectra of the enhanced juices and expressed as color retention percentage (CR% = $Abs_1 \times 100/Abs_0$) (**Figure 1A–D**). Immediately after copigment addition, an increase in color intensity was observed in the juices (data not shown). During storage, sinapic acid induced the strongest color in strawberry juice, ferulic and sinapic acids improved raspberry juice color equally, and rosmarinic acid enhanced the color of lingonberry and cranberry juices the most.

The color of the four plain nonenhanced berry juices was not stable during storage. The red color of strawberry juice was imperceptible after 36 days. Raspberry juice color was not measurable spectrophotometricly at λ_{max} in the red region after 51 days. The color of lingonberry and cranberry juices maintained its λ_{max} in the red region of the spectrum throughout storage, but the color intensity was only 33% of the original for lingonberry juice and 19% for cranberry juice in the end of storage.

All of the three phenolic acids enhanced strawberry juice color during storage (**Figure 1A**). Strawberry juice color was around 30% more intense when enhanced with phenolic acids than the nonenhanced juice color at a time point of 36 days. This enhancement increased during storage, being most efficient with sinapic acid after 75 days and thenceforth. The color of strawberry juice continued to intensify also with ferulic acid in the course of time. Rosmarinic acid enhanced strawberry juice color but less than the other two phenolic acids. The color of raspberry juice was enhanced most effectively with the two simple nonconjugated cinnamic acids, ferulic and sinapic acids (**Figure 1B**). In raspberry juice, sinapic and rosmarinic acids induced around 60% and ferulic acid induced around 35% more intense color than the plain nonenhanced juice at the time point of 51 days. Toward the end of storage, ferulic acid seemed to induce stronger color over sinapic acid, but the difference was not statistically significant. The enhancement of raspberry juice color by rosmarinic acid was not stable during storage even though it started as the strongest. The color of cranberry and lingonberry juices enhanced differently than the color of strawberry and raspberry juices. The color of lingonberry and cranberry juices was strongest throughout the storage period when enriched with rosmarinic acid (**Figure 1C**,**D**). Rosmarinic acid intensified cranberry juice color over 110% and lingonberry

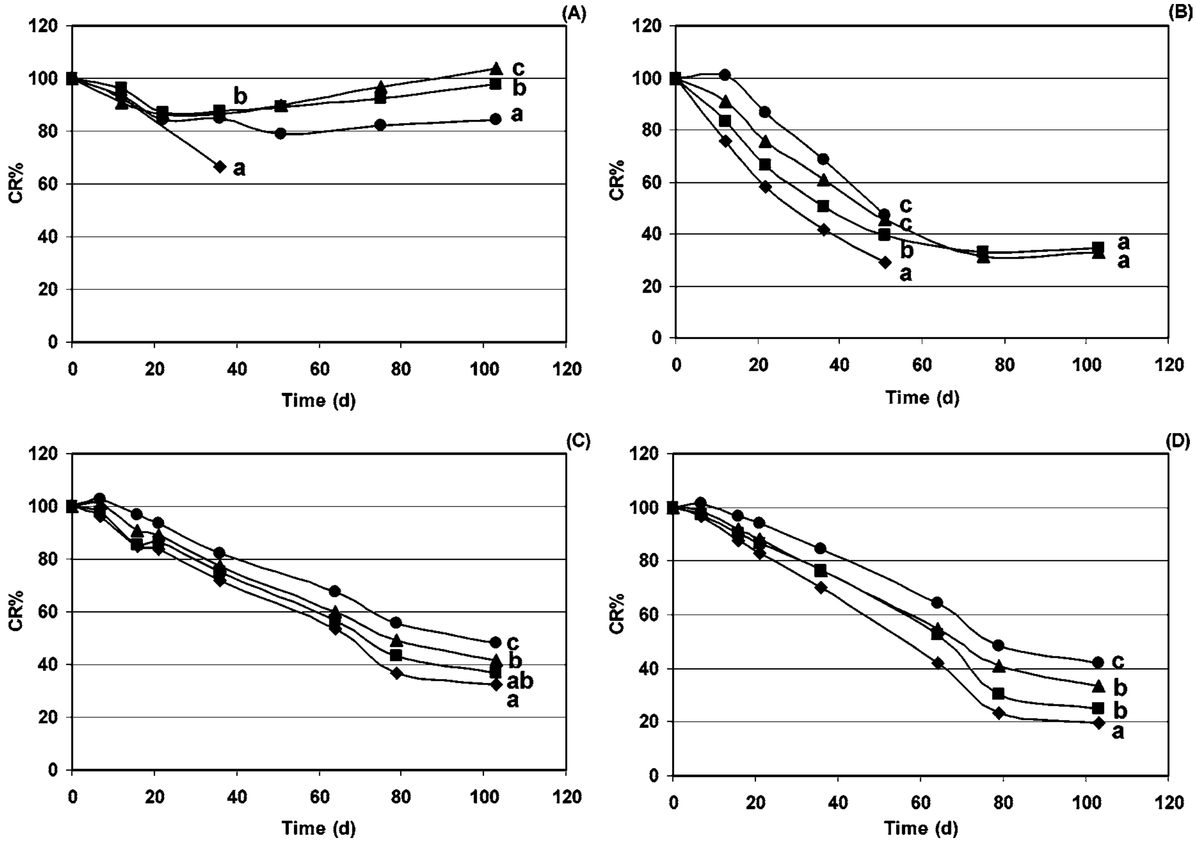


Figure 1. Color enhancement of berry juices by phenolic acids during storage detected as a change in the absorbance of λ_{max} and displayed as color retention percentage (CR%). Strawberry juice (**A**), raspberry juice (**B**), cranberry juice (**C**), and lingonberry juice (**D**). Plain nonenhanced juice, ◆; enhanced with ferulic acid, ■; enhanced with sinapic acid, ▲; and enhanced with rosmarinic acid, ●. Within the specific time point, values marked by the same letter are not significantly different.

juice color almost by 50% more than the color of the plain nonenhanced juice in the end of storage. Sinapic acid increased cranberry juice color by 70% and lingonberry juice color by 30% by the end of storage as compared to the plain nonenhanced juices at the same time point. Ferulic acid increased the juice colors by 30 and 10%, respectively.

The use of phenolic acids as copigments for all of the four juices improved their color stability. The plain strawberry juice lost its redness quickly during storage, but when copigmented with sinapic acid, the color intensity of strawberry juice in the end of storage was 104% of the original intensity of the nonenhanced juice. With added ferulic acid, the color of strawberry juice in the end of storage was 98% and with rosmarinic acid 84% of the original intensity of the nonenhanced juice color. With raspberry juice, the color stabilizing effect was more modest and observed with ferulic and sinapic acids. The former maintained 35% and the latter 33% of the original intensity of the nonenhanced raspberry juice color after 103 days. The color intensity of the plain nonenhanced cranberry juice decreased during storage by 80% and lingonberry juice by 67%. By the end of storage, cranberry juice copigmented with rosmarinic acid had 42% of the original color left, with sinapic acid 33% and with ferulic acid 25%, respectively. Lingonberry juice copigmented with rosmarinic acid had 48% of the original color left, with sinapic acid 42% and with ferulic acid 37%, respectively.

The commercial color enhancers improved the color of strawberry and raspberry juices immediately (**Figure 2A**,**B**). Grape skin extract immediately increased the color intensity of strawberry juice by 190% and black carrot extract by 166%. Grape skin extract enhanced the color of strawberry juice the most, but this color became brownish during storage and λ_{max} was not measurable spectrophotometricly in the scanned range after 51 days. This was also the case with Color'Plus, the immediate enhancement of which was modest (53%) in strawberry juice as compared to the other two commercial enhanceners. Grape skin extract induced the strongest color also in raspberry juice, immediately increasing the original juice color intensity by 70%. Black carrot extract increased raspberry juice color immediately by 58% and Color'Plus by 19%, respectively. Raspberry juice color enhanced with grape skin extract was more stable during storage than when enhanced with black carrot extract, in contrast to strawberry juice. In raspberry juice, the color intensity decreased only 56% when enhanced with grape skin extract, and with black carrot extract, it decreased 77% after 75 days of storage. The color intensity of raspberry juice enhanced with Color'Plus was not more stable than the plain nonenhanced juice even though the color itself was stronger; the λ_{max} was not measurable in the scanned range after 51 days.

CIELAB Analyses. The CIELAB system enables an approach to the changes of juice colors where the changes in hue, chromatic saturation, and overall lightness are taken into account. The perceptible and overall color impression of the berry juices depends on the relative amount of red and yellow color, which is expressed as an angle of hue (**Table 1**) in the CIELAB color space system. Hue angles are defined in color as 0/360° for magenta, 90° for yellow, 180° for green, and 270° for blue (*10, 11*). In the four plain nonenhanced berry juices, the hue values increased during storage, which denotes a more yellow tint development in the juices. The original plain strawberry juice was the most yellowish in the beginning of storage with a hue angle of 60°. The color of the original

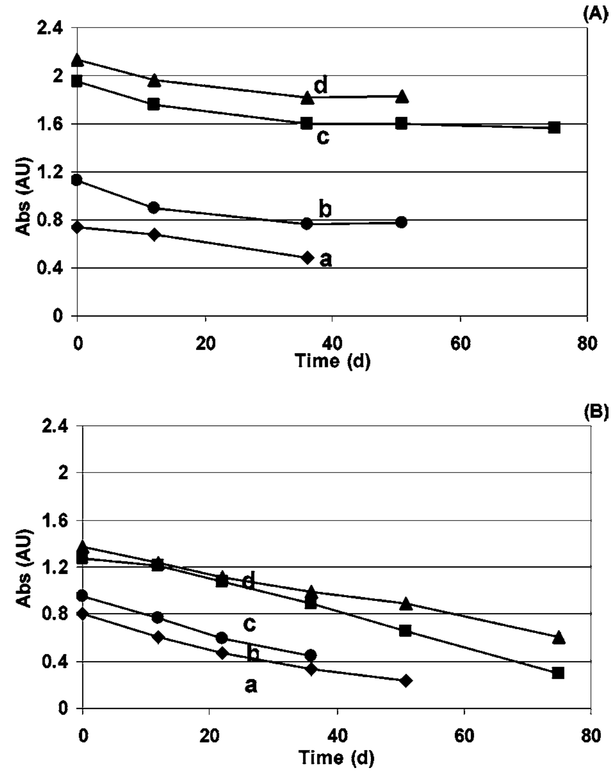


Figure 2. Color enhancement of strawberry juice (**A**) and raspberry juice (**B**) by commercial enhancers during storage detected as a change in the absorbance of λ_{max}. Plain nonenhanced juice, ◆; enhanced with grape skin extract, ▲; enhanced with black carrot extract, ■; and enhanced with rosmarinic extract, ●. Within the specific time point, all of the values were significantly different.

nonenhanced raspberry and cranberry juices was the reddest with a hue angle of 33°. The original nonenhanced lingonberry juice had a hue angle of 38° in the beginning of the study. The biggest change in hue angle was observed in raspberry juice, the hue angle of which increased 29° during storage. The hue angle of

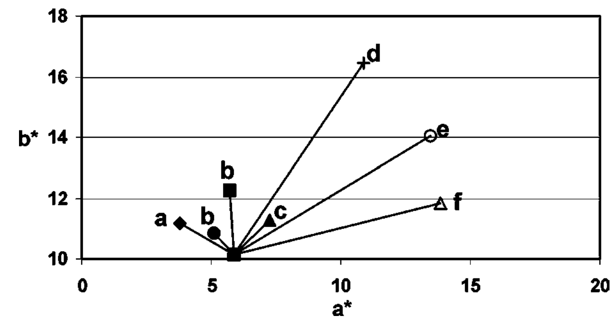


Figure 3. Changes of hue of strawberry juice with and without additions during storage expressed as a^*-coordinates (increasing redness) and b^*-coordinates (increasing yellowness). The starting point of the axis is the hue color coordinates of the plain nonenhanced strawberry juice in the beginning of storage. Plain nonenhanced strawberry juice, ◆; enhanced with ferulic acid, ■; enhanced with sinapic acid, ▲; enhanced with rosmarinic acid, ●; enhanced with grape skin extract, +; enhanced with black carrot extract, ○; and enhanced with rosmarinic extract, △. Values marked by the same letter are not significantly different.

the nonenhanced strawberry juice increased the least (11°). However, the final hue of the plain strawberry juice was the most yellow (71°) due to the highest hue angle of the juice in the beginning of storage. The smallest hue angle, i.e., the reddest color of the nonenhanced juices in the end of storage, was observed for both lingonberry and cranberry juices (48°).

The phenolic acid addition inhibited the expansion of the hue angle in all of the juices both immediately after supplementation and during storage. The hue angles in the beginning and after storage for all of the juices with or without addition are shown in **Table 1**. Sinapic acid was most efficient in this inhibition in all of the juices, especially in strawberry juice (**Figure 3**). In raspberry juice, sinapic and ferulic acids reduced the increment of the hue angle during storage, but rosmarinic acid did not have any effect. In cranberry and lingonberry juices, rosmarinic and sinapic acids were more effective than ferulic acid. The hue angle of strawberry and raspberry juices to which com-

Table 1. Chroma (C^*), Hue (h), and Color Difference (ΔE) of Berry Juices (X ± SD, $n = 4$)[a]

juice + copigment	chroma (C^*)		hue (h)		color difference (ΔE)	
time (days)	0[b]	103	0[b]	103	0[b]	103
strawberry	11.7 ± 0.3a	11.8 ± 1.0a	59.9 ± 0.2a	71.1 ± 3.0a		3.0 ± 0.7a
+ ferulic acid	12.0 ± 0.2a	13.5 ± 0.7bc	58.3 ± 0.2b	65.0 ± 0.5b	0.4 ± 0.1a	3.0 ± 0.6a
+ sinapic acid	12.2 ± 0.3a	13.4 ± 0.2abc	57.7 ± 0.2bc	57.2 ± 0.2c	0.8 ± 0.3a	2.4 ± 0.2a
+ rosmarinic acid	12.7 ± 0.2a	12.0 ± 0.2ab	57.2 ± 0.2c	64.7 ± 0.3b	1.2 ± 0.2ab	1.7 ± 0.2a
+ black carrot extract	20.6 ± 0.3b	18.2 ± 0.6d	32.7 ± 0.2e	40.5 ± 0.3d	13.3 ± 0.3c	10.0 ± 0.7c
+ grape skin extract	20.7 ± 0.5b	19.7 ± 0.8d	32.6 ± 0.2e	56.5 ± 0.1c	15.3 ± 0.6d	11.3 ± 0.9c
+ Color'Plus	12.7 ± 1.0a	14.7 ± 1.1c	55.9 ± 0.6d	72.0 ± 0.7a	1.9 ± 0.9b	5.1 ± 1.1b
raspberry	21.3 ± 2.5ab	10.4 ± 0.8a	33.4 ± 0.6a	62.5 ± 0.8d		12.7 ± 0.5a
+ ferulic acid	23.6 ± 1.1abc	11.6 ± 0.6ab	28.9 ± 0.3b	56.1 ± 0.7c	3.3 ± 1.0a	10.9 ± 0.4bc
+ sinapic acid	25.0 ± 1.0bc	11.7 ± 0.2ab	27.7 ± 0.2bc	51.4 ± 0.5b	5.2 ± 1.0ab	9.9 ± 0.2c
+ rosmarinic acid	25.7 ± 1.5c	12.0 ± 0.2bc	27.1 ± 0.2c	62.6 ± 1.6d	6.6 ± 0.2b	11.6 ± 0.3ab
+ black carrot extract	30.4 ± 1.9d	13.3 ± 0.6c	27.9 ± 0.1bc	57.3 ± 1.7c	11.1 ± 2.0c	9.6 ± 0.3cd
+ grape skin extract	32.1 ± 1.2d	22.9 ± 1.1d	24.7 ± 0.01d	47.3 ± 1.8a	15.5 ± 1.2d	8.5 ± 1.2d
+ Color'Plus	19.8 ± 2.6a	12.8 ± 0.8bc	27.7 ± 1.3bc	68.1 ± 2.9e	3.6 ± 1.0ab	12.5 ± 0.6a
lingonberry	39.5 ± 3.6	22.4 ± 0.9a	38.2 ± 0.0a	48.2 ± 0.1a		15.9 ± 1.2a
+ ferulic acid	42.2 ± 0.6	21.7 ± 2.8a	31.3 ± 0.1b	43.7 ± 1.2ab	6.2 ± 0.4a	15.8 ± 3.1a
+ sinapic acid	40.3 ± 3.6	25.7 ± 1.4ab	30.1 ± 0.3c	37.4 ± 0.6c	6.9 ± 0.9ab	10.8 ± 1.5b
+ rosmarinic acid	40.9 ± 4.1	28.5 ± 2.3b	28.9 ± 0.2d	40.2 ± 3.7bc	8.1 ± 0.8b	8.0 ± 2.8b
cranberry	37.8 ± 1.7	14.5 ± 0.7a	33.4 ± 0.1a	48.5 ± 1.9a		20.7 ± 0.9a
+ ferulic acid	38.2 ± 2.3	16.0 ± 0.5a	25.9 ± 0.1b	39.8 ± 0.3b	5.6 ± 0.3a	18.4 ± 0.5b
+ sinapic acid	36.8 ± 1.2	19.6 ± 1.4b	25.5 ± 0.2c	34.0 ± 4.2c	5.4 ± 0.1a	13.9 ± 1.5c
+ rosmarinic acid	39.4 ± 3.2	22.6 ± 0.9c	23.1 ± 0.1d	31.3 ± 0.5c	8.1 ± 0.9b	10.5 ± 1.0d

[a] Values marked by the same letter or no letter within the same column of the specific juice are not significantly different. [b] Time 0 days marks the immediate copigmentation effect observed after 2 h of enhancement addition.

Table 2. CIELAB Color Space Coordinates (L^*, a^*, and b^*) of Berry Juices (X ± SD, $n = 4$)[a]

juice + copigment	chromatic coordinates L^*		a^*		b^*	
time (days)	0[b]	103	0[b]	103	0[b]	103
strawberry	84.5 ± 0.1a	85.2 ± 0.1a	5.9 ± 0.2a	3.8 ± 0.2a	10.1 ± 0.3a	11.2 ± 1.1a
+ ferulic acid	84.4 ± 0.1a	84.4 ± 0.2ab	6.3 ± 0.2ab	5.7 ± 0.2c	10.2 ± 0.2a	12.3 ± 0.6a
+ sinapic acid	84.2 ± 0.2a	84.0 ± 0.1ab	6.5 ± 0.2abc	7.3 ± 0.1d	10.3 ± 0.3a	11.3 ± 0.2a
+ rosmarinic acid	84.1 ± 0.0a	84.7 ± 0.1ab	6.9 ± 0.2bc	5.1 ± 0.1bc	10.6 ± 0.2ab	10.8 ± 0.2a
+ black carrot extract	77.8 ± 0.2c	78.9 ± 0.3c	17.4 ± 0.3d	13.8 ± 0.5f	11.1 ± 0.2b	11.8 ± 0.4a
+ grape skin extract	74.5 ± 0.3d	77.4 ± 0.4c	17.4 ± 0.5d	10.9 ± 0.5e	11.1 ± 0.2b	16.4 ± 0.7c
+ Color'Plus	83.2 ± 0.5b	83.4 ± 0.6b	7.1 ± 0.7c	4.5 ± 0.2b	10.5 ± 0.8ab	14.0 ± 1.1b
raspberry	78.8 ± 1.3a	84.5 ± 0.3a	17.8 ± 2.2a	4.8 ± 0.3a	11.7 ± 1.2a	9.2 ± 0.8a
+ ferulic acid	77.2 ± 0.5ab	83.9 ± 0.3ab	20.6 ± 1.0ab	6.5 ± 0.3b	11.4 ± 0.5a	9.6 ± 0.5a
+ sinapic acid	76.2 ± 0.4b	83.2 ± 0.1bc	22.2 ± 0.9b	7.3 ± 0.2b	11.6 ± 0.4a	9.2 ± 0.2a
+ rosmarinic acid	76.1 ± 0.7b	83.4 ± 0.1b	22.9 ± 1.4b	5.5 ± 0.3a	11.7 ± 0.6a	10.7 ± 0.3ab
+ black carrot extract	72.9 ± 0.8c	82.3 ± 0.3c	26.9 ± 1.7c	7.2 ± 0.3b	14.2 ± 0.9b	11.2 ± 0.6b
+ grape skin extract	68.6 ± 0.6d	73.6 ± 0.9d	29.2 ± 1.0c	15.5 ± 0.8c	13.4 ± 0.5b	16.8 ± 1.1c
+ Color'Plus	77.9 ± 1.2ab	83.7 ± 0.4ab	17.6 ± 2.5a	4.7 ± 0.5a	9.2 ± 0.8c	11.9 ± 0.9d
lingonberry	71.4 ± 1.6	78.0 ± 0.6a	31.0 ± 2.9	14.6 ± 1.1a	24.4 ± 2.3a	16.9 ± 0.6ab
+ ferulic acid	68.9 ± 0.3	78.1 ± 1.5a	36.1 ± 0.5	15.7 ± 2.3a	21.9 ± 0.3ab	14.9 ± 1.6a
+ sinapic acid	69.6 ± 1.7	75.2 ± 0.8b	34.8 ± 3.1	20.4 ± 0.9b	20.2 ± 2.0b	15.6 ± 1.0a
+ rosmarinic acid	69.1 ± 2.0	73.8 ± 1.4b	35.8 ± 3.5	21.8 ± 2.8b	19.8 ± 2.1b	18.3 ± 0.8b
cranberry	71.3 ± 0.8	82.3 ± 0.5a	31.5 ± 1.5a	9.6 ± 0.8a	20.8 ± 0.9a	10.9 ± 0.4a
+ ferulic acid	70.1 ± 1.2	81.3 ± 0.3a	34.4 ± 2.0ab	12.3 ± 0.4b	16.7 ± 1.1b	10.2 ± 0.3a
+ sinapic acid	70.7 ± 0.6	78.6 ± 0.9b	33.2 ± 1.1ab	16.3 ± 1.9c	15.9 ± 0.5b	10.9 ± 0.6ab
+ rosmarinic acid	69.1 ± 1.6	76.7 ± 0.6c	36.3 ± 3.0b	19.3 ± 0.9d	15.4 ± 1.3b	11.8 ± 0.4b

[a] Values marked by the same letter or no letter within the same column of the specific juice are not significantly different. [b] Time 0 days marks the immediate copigmentation effect observed after 2 h of enhancement addition.

mercial color enhancements were added expanded during storage. Of the commercial enhancers, black carrot extract induced the least yellowing to strawberry juice and grape skin extract to raspberry juice.

The lightness (L^*) of color affects the appearance of a juice evidently. The L^* values in the beginning and after storage for all of the juices with and without addition are shown in **Table 2**. Phenolic acids affected the lightness of the berry juices somewhat during storage. In the beginning of storage, lingonberry and cranberry juices were much darker than raspberry juice, which again was noticeably darker than strawberry juice. The addition of colorless phenolic acids did not immediately affect the lightness of the juices noticeably. The addition of the anthocyanin containing extracts decreased the lightness of the initial berry juice color significantly making the juice darker than the original nonenhanced juice, which is obvious due to the increment of the pigment molecules. During storage, the juices with plant extracts lightened also.

The metric chroma (C^*) correlates for the saturation of color (*12*). The chromatic values in the beginning and after storage for all of the juices with or without addition are shown in **Table 1**. Chroma of the original plain juices decreased during storage except for strawberry juice, the chroma of which remained the same. The addition of rosmarinic acid reduced the decrement of chroma in raspberry, lingonberry, and cranberry juices. In strawberry juice, the addition of ferulic and sinapic acids increased the value of chroma after 103 days indicating a more vivid juice color development during storage. The final chromas of raspberry juice enhanced with the commercial extracts were lower than the chroma of the original plain raspberry juice. With strawberry juice, it was the opposite; the chromas of the juice enhanced with the plant extracts were higher than the original plain juice before storage.

The total color difference (ΔE), which is combined of the changes of the three components chroma, hue, and lightness, was greatest in cranberry juice and smallest in strawberry juice over the 103 days of storage. The total color differences in the beginning and after storage for all of the juices with or without addition are shown in **Table 1**. A threshold value of $\Delta E = 1$ is assumed as a basis for a color difference noticed by the human eye (*12*). In lingonberry and cranberry juices, the ΔE was the smallest in the juices enhanced with rosmarinic acid, and for that reason, rosmarinic acid evidently stabilized the color of these juices more than the other phenolic acids. In raspberry juice, there was a similar stabilizing effect observed with ferulic and sinapic acids but not with rosmarinic acid. In strawberry juice, ΔE did not reveal any statistically significant stabilization of the color, which is surprising since the changes of chroma and hue angle clearly indicate efficient color enhancement and stabilization by the phenolic acids as well as do the spectrophotometric results.

Table 3. Composition of the Different Berry Juices (X ± SD, $n = 3$)[a]

juice	pH	sugar (%)	total phenols (GAE g/L)	anthocyanins (mg/L)	vitamin C (mg/L)[b]
strawberry	3.5	10.4 ± 0.2	0.5 ± 0.004	38.6 ± 0.6	<0.3
raspberry	3.2	11.2 ± 0.1	0.4 ± 0.006	81.5 ± 0.5	<0.3
lingonberry	2.6	4.6 ± 0.1	1.3 ± 0.03	160.0 ± 0.8	<0.3
cranberry	2.5	3.0 ± 0.1	0.8 ± 0.01	140.0 ± 0.3	<0.3

[a] Values within the same column are significantly different. [b] The vitamin C contents were below the detection limit.

Juice Compositions. The basic compositions of the juices were measured, including the total phenolic and soluble solids content, pH, anthocyanin content, and the vitamin C content (**Table 3**). Strawberry juice contained 0.5 g/L of total phenols expressed as GAE; raspberry juice, 0.4 g/L; lingonberry, 1.3 g/L; and cranberry, 0.8 g/L, respectively. The sugar content that was determined as soluble solids (Brix°) of the juices was 10% for strawberry juice, 11% for raspberry juice, 5% for lingonberry juice, and 3% for cranberry juice. The commercial strawberry and raspberry juices were fortified with sugar, but sugar was not added to the self-made lingonberry and cranberry juices. The pH values of the juices were quite similar ranging between

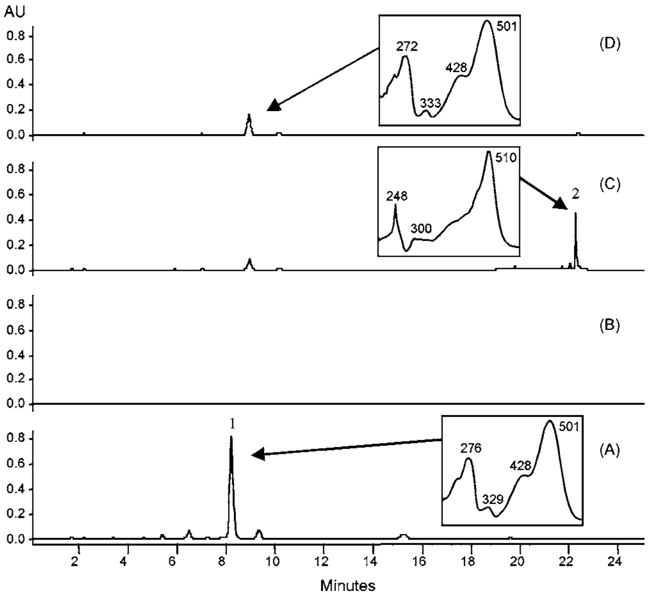


Figure 4. HPLC chromatograms of strawberry juice during storage with and without phenolic acid addition measured at 520 nm with UV–vis spectra. Plain nonenhanced strawberry juice in the beginning of storage (**A**); plain nonenhanced strawberry juice in the end of storage (**B**); strawberry juice enhanced with sinapic acid in the end of storage (**C**); and strawberry juice enhanced with rosmarinic acid in the end of storage (**D**). The injection volume was 100 μL for all. Peak 1, pelargonidin 3-glucoside; peak 2, novel copigmentation compound.

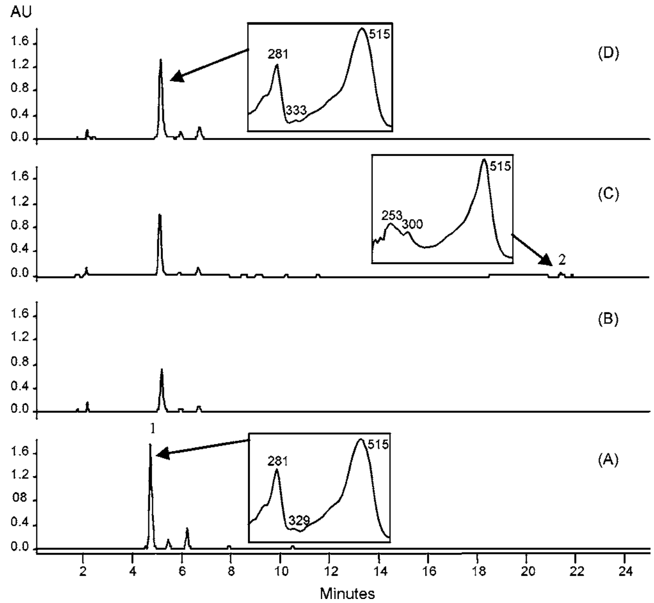


Figure 5. HPLC chromatograms of lingonberry juice during storage with and without phenolic acid addition measured at 520 nm with UV–vis spectra. Plain nonenhanced lingonberry juice in the beginning of storage (**A**); plain nonenhanced lingonberry juice in the end of storage (**B**); lingonberry juice enhanced with sinapic acid in the end of storage (**C**); and lingonberry juice enhanced with rosmarinic acid in the end of storage (**D**). The injection volume was 30 μL for **A** and 100 μL for all of the others. Peak 1, cyanidin 3-galactoside; peak 2, novel copigmentation compound.

2.5 and 3.5. Strawberry juice had the highest pH (3.5), the pH of raspberry juice was a little lower (3.2), the pH of lingonberry juice was lower (2.6) than the two commercial juices, and cranberry juice was the most acidic of the juices with a pH of 2.5. The vitamin C content of the juices was also measured in order to estimate its influence on anthocyanin stability and the copigmentation reactions. However, the vitamin C content of all of the juices was below the detection limit of 0.3 mg/L and can be assumed to have none or only a remote effect on the color enhancement.

Changes in Anthocyanin Profile (HPLC) during Storage. The total anthocyanin content of all of the juices decreased during storage, as shown by the reduction in total peak area measured at 520 nm. In the beginning of the study, lingonberry juice had the highest anthocyanin content (0.16 g/L), cranberry juice the second highest (0.14 g/L), strawberry juice the lowest (0.04 g/L), and raspberry juice the second lowest (0.08 g/L) (**Table 3**). After 103 days of storage, only very small amounts of anthocyanins were left in the plain nonenhanced juices. Lingonberry juice sustained the highest anthocyanin content after storage, which was 13% of the original amount. Ninety percent of cranberry juice anthocyanins were lost during storage. Raspberry and strawberry juices had lost almost all of their anthocyanins, containing only 1% of them in the end of storage.

Enriching the juices with phenolic acids produced novel peaks to the end of HPLC chromatogram indicating formation of new compounds during storage. The number of the new forming compounds was in relation to the number of original individual anthocyanin compounds in each juice. The most abundant number of novel compounds was discovered in cranberry juice. In the chromatogram of strawberry juice, which contained one major anthocyanin, pelargonidin 3-glucoside, one novel major compound arose (**Figure 4C**). With strawberry juice, the area of these peaks grew with time. This increment was not observed with the other juices, however, but neither did any other juice sustain its color as efficaciously when enhanced with phenolic acids than strawberry juice.

Each phenolic acid induced these novel compounds differently with the four juices. Sinapic acid produced the most abundant peaks with strawberry juice. With raspberry juice, ferulic acid induced the most pronounced peaks. The reactions in lingonberry and cranberry juices with phenolic acids differed from the two former juices. Sinapic and ferulic acids produced only modest peaks with them. Surprisingly, rosmarinic acid acted quite differently as compared to the other two phenolic acids. Rosmarinic acid stabilized lingonberry and cranberry juice anthocyanins but did not induce new peaks in their chromatograms (**Figure 5D**). The anthocyanin content of lingonberry juice diminished only 72% when rosmarinic acid was added. With sinapic acid, it decreased 78% and with ferulic acid 84%. With strawberry and raspberry juices, rosmarinic acid had a lesser effect. With sinapic acid addition, the total amount of anthocyanins in strawberry juice diminished only by 74%, 84% with ferulic acid, and 81% with rosmarinic acid.

Enrichment of strawberry and raspberry juices with commercial color enhancers, grape skin extract and black carrot extract, also produced new peaks to the chromatograms. However, this is due to characteristic anthocyanins of the color enhancers. The third commercial color enhancer, Color'Plus, which is a nonflavor rosemary extract but does not contain anthocyanins itself, induced only one new peak to raspberry juice in the beginning of the study. In the end of storage, there were very modest new peaks in the chromatogram of strawberry juice enhanced with Color'Plus and no significant ones in raspberry juice.

DISCUSSION

Color Enhancement. The addition of phenolic acids to the four different berry juices improved the juice color by enhancing the anthocyanin color intensity and stabilizing the color during storage. Nonenhanced raspberry and strawberry juices lost their anthocyanin pigments and redness quickly during storage. The color intensity of nonenhanced lingonberry and cranberry juices decreased also during storage, even though this was slower than with strawberry and raspberry juices. Vigorous decrement of anthocyanin content of different berry products during storage have been reported before for strawberry preserves (*13*), strawberry and black currant syrups (*14*), strawberry juice (*15*), red radish and red-fleshed potato juices (*16*), beverages containing sweet potato anthocyanins (*17*) and grape anthocyanins (*18*), blood orange juice concentrate (*19*), raspberry pulp (*20*), raspberry juice concentrate (*21*), and cranberry juice cocktail (*22*). However, enhancement and stabilization of the color of berry juices by phenolic acids during storage have not been studied previously; hence, novel information on anthocyanin enhancement and copigmentation in food models is provided here. The stability of the color of berry products has been investigated in the past concentrating on the effects of processing methods (*6*, *23*−*27*), packaging material and added preservatives (*28*), and the addition of sugar (*29*) and ascorbic acid (*14*). The effects of flavonol addition on anthocyanin color in model systems (*30*) and in blood orange juice (*5*) have also been studied before. In these studies, flavonols, i.e., rutin and quercetin but also tartaric and caffeic acids, reduced the loss of anthocyanins. These results support our findings and hypothesis of a protective effect of phenols, especially phenolic acids, on anthocyanin color.

Juice Preparation Effects. It is well-known that heat treatment such as pasteurization decreases anthocyanin content of juices (*6*, *7*, *15*). Therefore, we tried to find a gentle way to sterilize the berry juices and to avoid any additional deterioration. Sterilization through 0.2 μm sterile filters, however, was not completely uniform and resulted in an uneven batch. Sterilization in a microwave was also uneven and resulted in inconsistent batches. Using chemical compounds such as NaN_3 for sterilization is not permitted in foods. They also take part in undesired chemical reactions, which affect the desired copigmentation reactions. The only gentle sterilization method suitable for our study was an ultrasonic bath for 45 min. This presumably broke down the cell walls of unwanted bacteria and yeast but did not noticeably affect the anthocyanin or other phenolic molecules.

It is believed that copigmentation reactions in wine are dependent on fermentation. The color enhancement by different phenols has been previously reported concerning mostly only red wine (*31*, *32*). Darias-Martin et al. (*33*) noticed that the color of young red wine to which caffeic acid was added before fermentation maintained its enhancement during storage. Here, however, we showed that anthocyanin copigmentation reactions do not require fermentation and take place also in nonfermented sterile berry juices.

Differently Reacting Berry Juices. The four juices can be classified into two different groups by their composition but also by their color behavior and copigmentation reactions during storage. Strawberry and raspberry juices contained less phenolics and anthocyanins than cranberry and lingonberry juices, the amounts of which are in accordance with previous studies on berry phenolics (*34*, *35*). Also, the pH and sugar content were significantly higher in the two former juices as compared to the latter ones. The different anthocyanin profiles of the juices are of course one of the main reasons for the different copigmentation and enhancement reactions. Strawberry and raspberry have similar anthocyanin profiles and correspondingly so do lingonberry and cranberry (*35*−*38*), which is also evident from the HPLC data. The copigmentation reactions were similar within strawberry and raspberry juices, the reactions of which differed from the copigmentation reactions perceived in lingonberry and cranberry juices, which again were similar to one another. Strawberry juice was most responsive to copigmentation, the color of which was enhanced the most by the phenolic acids. Raspberry juice was comparatively receptive to color enhancement, and the color development observed in lingonberry and cranberry juices was more modest. Garzon and Wrolstad (*39*) came to the conclusion when studying the stability of strawberry juice and concentrate that the higher the total pigment concentration the more stable the berry juice or product, which is in an agreement with our results on overall anthocyanin stability. Skrede et al. (*14*) stated that color stability is more dependent on the total anthocyanin content rather than the qualitative anthocyanin composition. This is true for overall color stability, but when it comes to color enhancement, especially through copigmentation reactions, the results are very much dependent on the individual anthocyanin composition, as shown here. In our previous study with pure compounds (*4*), copigmentation occurred more intensively with pelargonidin 3-glucoside, the main anthocyanin of strawberry, than with cyanidin 3-glucoside, which is one of the main anthocyanins of raspberry. These same observations were made in this study with the juices substantiating also that copigmentation reactions, which were seen with pure compounds, can be accomplished also in food matrix.

Effect of Phenolic Acids. The phenolic acids used can also be classified into two groups by their chemical structure for one but also by their manifestation of copigmentation in the juices. Sinapic and ferulic acids induced the strongest hyperchromic effects to strawberry and raspberry juices. They also formed new intramolecular copigmentation molecules with strawberry and raspberry anthocyanins, which were detected as novel peaks in the HPLC chromatograms. Presumably, the new molecules are a result of covalent bonding between an anthocyanin and a phenolic acid since they endure the acidic HPLC conditions.

The enhancement by sinapic and ferulic acids was not as strong with lingonberry and cranberry anthocyanins; instead, it was perceived that rosmarinic acid affected the color stability of these juices most efficiently. Rosmarinic acid obviously stabilized lingonberry and cranberry anthocyanins via intermolecular copigmentation reactions since their color was stabilized and the diminishing of anthocyanins was reduced, but no new anthocyanin compounds were detected with HPLC.

Also, the different pK_a values of the phenolic acids can affect the stabilization of the berry juice colors. The pK_a values of ferulic and sinapic acids are around 4.6. The pK_a of rosmarinic acid is 2.8, which indicates that in the pH of lingonberry and cranberry juices most of rosmarinic acid is dissociated, unlike in raspberry and strawberry juices. Obviously, the negatively charged rosmarinic acid protects anthocyanins effectively in the more acidic juices. This difference in chemical behavior between the conjugated cinnamic acid, rosmarinic acid, and the simple cinnamic acids, ferulic acid and sinapic acid with anthocyanins, was also shown in our previous study with pure compounds (*4*). Schwarz et al. (*40*) suggested the formation of anthocyanin−vinylphenol adducts in red wines through enzymatic decarboxylation, and perhaps, similar compounds were also formed here between berry juice anthocyanins and the simple cinnamic acids. In our previous study, methoxylated cinnamic acid, ferulic acid, was a better copigment over nonmethoxylated cinnamic acid, caffeic acid, with pelargonidin 3-glucoside. In strawberry juice, sinapic acid, the dimethoxylated cinnamic acid, was a more a efficient color enhancer than ferulic acid; thus, it can be concluded that the increasing methoxylation of the simple cinnamic acids lubricates the formation of copigmentation complexes in juices containing mainly monoglycosidic anthocyanins.

The effects on color stabilization and copigmentation of the commercial color enhancers on the berry juices were not as adequate as the corresponding effects of the phenolic acids. Although the initial intensifying of the berry juice color by the extracts was strong, the stability of the color was poor, except with black carrot extract in strawberry juice. The introduction of new anthocyanins and the increment of the anthocyanin amount itself stabilized the juice colors somewhat.

CIELAB Color Space System. When it comes to berry juice color, color enhancement, and stability, the picture is incomplete if only looking at the λ_{max} of the absorption spectrum of a juice. Therefore, it was important to monitor the total color change and different aspects affecting the manifestation of berry juice color with the CIELAB color space system, which enables an approach to the changes of juice colors where the changes in hue, chromatic saturation, and overall lightness are taken into account.

The total color changes (ΔE) of the juices with and without color enhancers showed clearly that the changes in the berry juice colors during storage were visible also to the naked eye. During storage, all of the juices yellowed, which was indicated by the increment of hue angle. This is in accordance with previous studies where strawberry juice (*15*), preserves (*13*), and blackcurrant syrups (*14*) obtained higher hue angles and became more yellow during storage. The hue angle of strawberry juice enhanced with ferulic acid in the end of storage was significantly greater than the one of the plain nonenhanced juice in the end of storage (**Figure 3**), and yet, the a^* value, standing for the redness, was almost the same. Therefore, the a^* value is not sufficient enough for interpretation of the stability of berry juice color. For example, the juice color of strawberry juice enhanced with ferulic acid can be considered red but it has yellowed during storage since the b^* value has increased significantly and the hue angle has increased. Bathochromic shift of a juice does not enlighten the change of tint sufficiently enough either. Because the bathochromic shift marks only the change of the absorption maximum (λ_{max}) of a spectrum, it disregards the effects of other wavelengths, which are also important in the definition of the overall berry juice color. In this study, the benefit of measuring the CIELAB coordinates is shown most clearly in the case of raspberry juice, the color of which was undeterminable as λ_{max} in the red region after storage time of 51 days, and yet, the hue values on the CIELAB scale show that raspberry juices with and without additions have reddish color after a storage time of 103 days.

In conclusion, the reactions observed with the four berry juices and added enhancers differed significantly by their mechanisms and manifestations. Strawberry and raspberry juices were the most receptive juices to color enhancement, and acidic lingonberry and cranberry juices were the least receptive, and yet, the original anthocyanins were stabilized most efficiently in the two latter juices. Intramolecular copigmentation reactions are most likely responsible for the enhancement of raspberry and strawberry juice color by simple cinnamic acids, sinapic and ferulic acid, and intermolecular copigmentation for the conjugated cinnamic acid, rosmarinic acid. The identification of the formed novel intramolecular copigmentation complexes is the next challenge in the elucidation of the enhancement and stabilization of berry juice color.

LITERATURE CITED

(1) Markakis, P. Stability of anthocyanins in foods. In *Anthocyanins as Food Colors*; Markakis, P., Ed.; Academic Press Inc.: London, U.K., 1982; pp 163−180.

(2) Deighton, N.; Brennan, R.; Finn, C.; Davies, H. V. Antioxidant properties of domesticated and wild Rubus species. *J. Sci. Food Agric.* **2000**, *80*, 1307−1313.

(3) Hollman, P. C. H.; Hertog, M. G. L.; Katan, M. B. Role of dietary flavonoids in protection against cancer and coronary heart disease. *Biochem. Soc. Trans.* **1996**, *24*, 785−789.

(4) Eiro, M. J.; Heinonen, M. Anthocyanin color behavior and stability during storage: effect of intermolecular copigmentation. *J. Agric. Food Chem.* **2002**, *50*, 7461−7466.

(5) Maccarone, E.; Maccarone, A.; Rapisarda, P. Stabilization of anthocyanins of blood orange fruit juice. *J. Food Sci.* **1985**, *50*, 901−904.

(6) Talcott, S. T.; Brenes, C. H.; Pires, D. M.; Del Pozo-Insfran, D. Phytochemical Stability and Color Retention of Copigmented and Processed Muscadine Grape Juice. *J. Agric. Food Chem.* **2003**, *51*, 957−963.

(7) Bakker, J.; Bridle, P. Strawberry juice color: the effect of sulfur dioxide and EDTA on the stability of anthocyanins. *J. Sci. Food Agric.* **1992**, *60*, 477−481.

(8) Poei-Langston, M. S.; Wrolstad, R. E. Color degradation in an ascorbic acid-anthocyanin-flavanol model system. *J. Food Sci.* **1981**, *46*, 1218−1222, 1236.

(9) Wrolstad, R. E.; Erlandson, J. A. Effect of metal ions on the color of strawberry puree. *J. Food Sci.* **1973**, *38*, 460−463.

(10) Gonnet, J.-F. Color effects of co-pigmentation of anthocyanins revisited-2. A colorimetric look at the solutions of cyanin co-pigmented by rutin using the CIELAB scale. *Food Chem.* **1999**, *66*, 387−394.

(11) Price, C. L.; Wrolstad, R. E. Anthocyanin pigments of Royal Okanogan huckleberry juice. *J. Food Sci.* **1995**, *60*, 369−374.

(12) Gonnet, J.-F. Color effects of co-pigmentation of anthocyanins revisited − 1. A colorimetric definition using the CIELAB scale. *Food Chem.* **1998**, *63*, 409−415.

(13) Abers, J. E.; Wrolstad, R. E. Causative factors of color deterioration in strawberrt preserves during processing and storage. *J. Food Sci.* **1979**, *44*, 75−78, 81.

(14) Skrede, G.; Wrolstad, R. E.; Lea, P.; Enersen, G. Color stability of strawberry and black currant syrups. *J. Food Sci.* **1992**, *57*, 172−177.

(15) 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.

(16) Rodriguez-Saona, L. E.; Giusti, M. M.; Wrolstad, R. E. Color and pigment stability of red radish and red-fleshed potato anthocyanins in juice model systems. *J. Food Sci.* **1999**, *64*, 451−456.

(17) Bassa, I. A.; Francis, F. J. Stability of anthocyanins from sweet potatoes in a model beverage. *J. Food Sci.* **1987**, *52*, 1753−1754.

(18) Palamidis, N.; Markakis, P. Stability of grape anthocyanin in carbonated beverages. *J. Food Sci.* **1975**, *40*, 1047−1049.

(19) Kirca, A.; Bekir, C. Degradation kinetics of anthocyanins in blood orange juice and concentrates. *Food Chem*. **2003**, *81*, 583−587.

(20) Ochoa, M. R.; Kesseler, A. G.; Vullioud, M. B.; Lozano, J. E. Physical and chemical characteristics of raspberry pulp: storage effect on composition and color. *Lebensm.-Wiss. Technol.* **1999**, *32*, 149−153.

(21) Withy, L. M.; Nguyen, T. T.; Wrolstad, R. E.; Heatherbell, D. A. Storage changes in anthocyanin content of red raspberry juice concentrate. *J. Food Sci.* **1993**, *58*, 190−192.

(22) Starr, M. S.; Francis, F. J. Effect of metallic ions on color and pigment content of cranberry juice cocktail. *J. Food Sci.* **1973**, *38*, 1043−1046.

(23) Zabetakis, I.; Leclerc, D.; Kajda, P. The effect of high hydrostatic pressure on the strawberry anthocyanins. *J. Agric. Food Chem.* **2000**, *48*, 2749−2754.
(24) 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.
(25) Rommel, A.; Wrolstad, R. E.; Heatherbell, D. A. Blackberry juice and wine: processing and storage effects on anthocyanin composition, color and appearance. *J. Food Sci.* **1992**, *57*, 385−391, 410.
(26) Rommel, A.; Heatherbell, D. A.; Wrolstad, R. E. Red raspberry juice and wine: effect of processing and storage on anthocyanin pigment composition, color and appearance. *J. Food Sci.* **1990**, *55*, 1011−1017.
(27) Jiang, J.; Paterson, A.; Piggott, J. R. Effects of pectolytic enzyme treatments on anthocyanins in raspberry juice. *Int. J. Food Sci. Technol.* **1990**, *25*, 596−600.
(28) Thakur, B. R.; Arya, S. S. Studies on stability of blue grape anthocyanins. *Int. J. Food Sci. Technol.* **1989**, *24*, 532−326.
(29) Wrolstad, R. E.; Skrede, G.; Lea, P.; Enersen, G. Influence of sugar on anthocyanin pigment stability in frozen strawberries. *J. Food Sci.* **1990**, *55*, 1064−1065, 1072.
(30) Shrikhande, A. J.; Francis, F. J. Effect of flavonols on ascorbic acid and anthocyanin stability in model systems. *J. Food Sci.* **1974**, *39*, 904−906.
(31) Darias-Martin, J.; Martin-Luis, B.; Carrillo-Lopez, M.; Lamuela-Raventos, R.; Diaz-Romero, C.; Boulton, R. Effect of Caffeic Acid on the Color of Red Wine. *J. Agric. Food Chem.* **2002**, *50*, 2062−2067.
(32) Mateus, N.; Silva, A. M. S.; Vercauteren, J.; de Freitas, V. Occurrence of Anthocyanin-Derived Pigments in Red Wines. *J. Agric. Food Chem.* **2001**, *49*, 4836−4840.
(33) Darias-Martin, J.; Carrillo, M.; Diaz, E.; Boulton, R. B. Enhancement of red wine colour by pre-fermentation addition of copigments. *Food Chem.* **2001**, *73*, 217−220.
(34) Kähkönen, M. P.; Hopia, A. I.; Heinonen, M. Berry phenolics and their antioxidant activity. *J. Agric. Food Chem.* **2001**, *49*, 4076−4082.
(35) Prior, R. L.; Lazarus, S. A.; Cao, G.; Muccitelli, H.; Hammerstone, J. F. Identification of procyanidins and anthocyanins in blueberries and cranberries (*Vaccinium Spp*) using high-preformance liquid chromatography/mass spectrometry. *J. Agric. Food Chem.* **2001**, *49*, 1270−1276.
(36) Kähkönen, M. P.; Heinimäki, J.; Ollilainen, V.; Heinonen, M. Berry anthocyanins: isolation, identification and antioxidant activities. *J. Sci. Food Agric.* **2003**, *83*, 1403−1411.
(37) Lopes-Da-Silva, F.; De Pascual-Teresa, S.; Rivas-Gonzalo, J.; Santos-Buelga, C. Identification of anthocyanin pigments in strawberry (*cv Camarosa*) by LC using DAD and ESI-MS detection. *Eur. Food Res. Technol.* **2002**, *214*, 248−253.
(38) De Ancos, B.; Gonzalez, E.; Cano, M. P. Differentiation of raspberry varieties according to anthocyanin composition. *Z. Lebensm. Unters. Forsch. A* **1999**, *208*, 33−38.
(39) 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.
(40) Schwarz, M.; Wabnitz, T. C.; Winterhalter, P. Pathway leading to the formation of anthocyanin-vinylphenol adducts and related pigments in red wines. *J. Agric. Food Chem.* **2003**, *51*, 3682−3687.

Received for review December 23, 2003. Revised manuscript received February 19, 2004. Accepted March 9, 2004. This work was supported by the Commission of the European Communities, Quality of life and management of living sources specific RTD program QLK1-1999-00124 "Functional Properties, Bioactivities and Bioavailability of Phytochemicals, Especially Anthocyanins, from Processed Foods". It does not necessarily reflect its views and in no way anticipates the Commission's future policy in this area.

JF035507I