Influence of Processing and Storage on the Phenolic Composition of Thompson Seedless Grape Juice†

George A. Spanos and Ronald E. Wrolstad*

Department of Food Science and Technology, Oregon State University, Corvallis, Oregon 97331

Methodology for measurement of phenolics in fruit juices using HPLC separation and diode array detection is presented. Quantitation of phenolic acids and flavonol glycosides was achieved with minimum sample preparation. Procyanidin quantitation, however, required removal of interfering compounds with Sephadex LH-20 minicolumn chromatography. Good reproducibility and high recoveries (up to 92.3%) were achieved in procyanidin isolation. The methods were used to study the effect of SO_2, enzymatic clarification, fining, bottling, concentration, and storage on the phenolic composition of Thompson Seedless grape juice. SO_2 addition during processing resulted in higher levels of phenolic acids and procyanidins, but it had no apparent effect on the quercetin glycoside composition. Oxidation of caftaric acid to 2-*S*-glutathionylcaftaric acid was evident in juices processed both with and without SO_2. Enzymatic clarification caused hydrolysis of caftaric, coutaric, and quercetin derivatives, but it showed no effect on the 2-*S*-glutathionylcaftaric acid. Procyanidins demonstrated sensitivity to the heat applied during bottling and concentration. Storage of concentrates for 9 months at 25 °C led to the formation of (hydroxymethyl)furfural (HMF) (up to 33.5 mg/L), extensive oxidation of cinnamics, and total loss of procyanidins and quercetin glycosides. Colorimetric measurement of phenolics showed no correlation with the HPLC quantitation.

INTRODUCTION

The complexity of the phenolic composition and the importance of phenolic constituents in color, flavor, and stability characteristics of grape juices and wines are well recognized (Singleton and Esau, 1969). It is also known that variety, processing practices, and storage, among other factors, affect the phenolic composition of grape juices and wines (Amerine and Ough, 1980).

Previous work has shown that the tartaric esters of caffeic and coumaric acids are the major cinnamics (Ribéreau-Gayon, 1965; Singleton et al., 1978; Baranowski and Nagel, 1981). Procyanidins built on epicatechin and catechin units make up the procyanidin fraction (Lea et al., 1979) of grape juices and wines. Flavonol derivatives (Cheynier and Rigaud, 1986) and hydroxybenzoic acids (Singleton and Trousdale, 1983) also occur. Colorimetric procedures and paper or thin-layer chromatography that have been used in analysis of phenolics often give semiquantitative data. HPLC procedures have also been used recently, but they present the difficulties of interpretation of complex chromatograms and the possibility of coelution (Singleton and Trousdale, 1983). Recent advances in diode array detection are useful not only in phenolic identification but also in their quantitation by checking peak purity or by simultaneously recording more than one wavelength. Most of the efforts in the research of grape phenolics has focused on varieties and processing techniques associated with the production of wines and less emphasis has been given to varieties and processing of grapes for production of juice.

This paper presents methodology that utilizes reverse-phase HPLC and diode array detection to profile the phenolic composition of fruit juices. The influence of processing and storage on the phenolic composition of Thompson Seedless grape juice is investigated. Comparison between HPLC and colorimetric quantitation of phenolics is also made.

MATERIALS AND METHODS

Standards. Phenolic standards (chlorogenic, caffeic, *p*-coumaric, gallic, and protocatechuic acids, rutin, catechin, and epicatechin), tryptophan, tyrosine, and (hydroxymethyl)-furfural (HMF) were obtained from Sigma Chemical Co. (St. Louis, MO). Procyanidins (B1, B2, B3, B4, trimer, and tetramer) were provided by Dr. A. Lea, Cadbury Schweppes Ltd., Lord Zuckerman Research Center, University of Whiteknights, U.K. Caftaric and coutaric acids were donated by Dr. V. L. Singleton, Department of Enology, University of California, Davis, CA. All solvents used in this investigation were of HPLC grade.

Grape Juice Processing. Thompson Seedless grapes obtained in fall 1986 from Papagni Vineyards, Clovis, CA, were processed into juice in the pilot plant of the Department of Food Science at Oregon State University, according to the flowsheet diagram shown in Figure 1. All processing trials were replicated. Juice samples were obtained in the intermediate processing steps as indicated in Figure 1 and stored frozen at −30 °C until analysis. Concentrates that had been kept frozen or stored for 9 months at 25 °C were diluted to their original degrees Brix before analysis. Detailed information about the processing is described elsewhere (Wrolstad et al., 1989).

Determination of Grape Phenolics with HPLC. All determinations were replicated, the mean values being reported; reproducibility was ca. ±5%.

Sample Preparation. For determination of phenolic acids and flavonol glycosides, single-strength grape juice was filtered through a Millipore 0.45-μm filter (type HA) and injected onto the HPLC.

Procyanidins were isolated by using Sephadex LH-20 (Pharmacia, Uppsala, Sweden). One gram was swelled in water and

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* To whom correspondence should be addressed.

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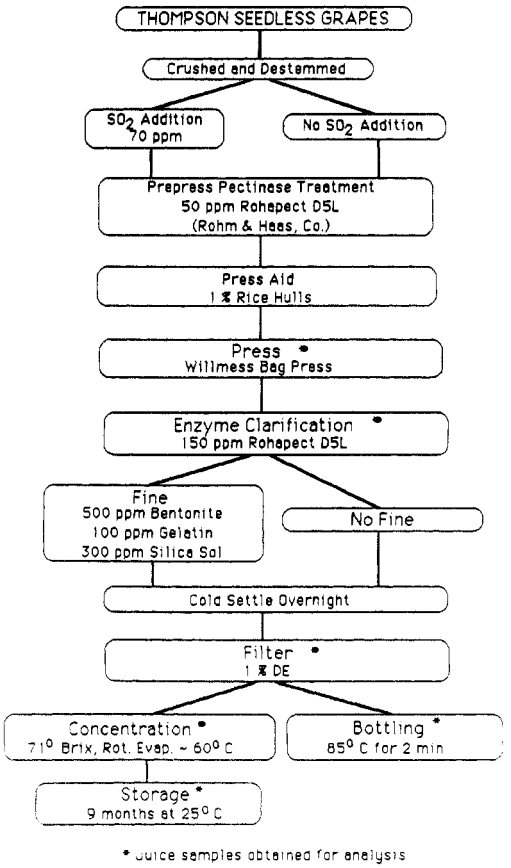

Figure 1. Outline of steps utilized in processing of Thompson Seedless grapes.

slurry-packed into a disposable Poly-Prep chromatography column (graduated column, 0.8 × 4 cm, holding up to 2 mL of chromatographic media and including an integral 10-mL reservoir, Bio-Rad Laboratories). The bed was rinsed with 5 mL of distilled water. Grape juice (10 mL) was carefully applied onto the column, and the bed was washed with 30 mL of 20% methanol. This fraction contained sugars, nonvolatile acids, and phenolic acids and was discarded. The procyanidins were eluted from the column with 15 mL of methanol. Percolation through the column was assisted with the application of a slight vacuum. The procyanidin fraction was evaporated to dryness on a rotary evaporator (water bath 45 °C), dissolved in 2 mL of distilled water, filtered through a Millipore 0.45-μm (type HA) filter, and injected onto the HPLC.

Analysis and Quantitation. The analytical equipment consisted of a Perkin-Elmer Series 400 liquid chromatograph, a Hewlett-Packard 1040A photodiode array detector interfaced with a Hewlett-Packard 9000 computer, a Beckman 501 autosampler with 50-μL injection loop, and a Supelcosil LC-18, (5 μm), 25 cm × 4.6 mm i.d., analytical column fitted with an ODS-10, 4 cm × 4.6 mm i.d., Micro-Guard column (Bio-Rad). Elution was carried out at room temperature and utilized as solvent A 0.07 M KH_2PO_4 adjusted to pH 2.5 with concentrated phosphoric acid and as solvent B, methanol. The elution program was, at a flow rate of 1 mL/min, 2% solvent B isocratic for 5 min followed by a 2–40% linear gradient with solvent B for 40 min and holding with 40% solvent B for an additional 12 min. The chromatogram was monitored simultaneously at 280 and 320 nm with 6-nm bandwidth, with spectra taken frequently during peak detection. Analytical conditions were the same for both the phenolic acids and the procyanidin fraction.

Calculation of concentrations was based on the external standard method. Dilutions 1:0, 1:2, 1:4, and 1:6 of a water solution containing 60 mg/L chlorogenic acid, 25 mg/L caffeic acid, 20 mg/L *p*-coumaric acid, 10 mg/L ferulic acid, 15 mg/L protocatechuic acid, 10 mg/L gallic acid, 10 mg/L rutin, 25 mg/L HMF, 25 mg/L tyrosine, and 20 mg/L tryptophan were used to fit a standard curve (area vs concentration in milligrams per liter) with linear regression for each individual compound. *cis*- and *trans*-caftaric acids, *cis*- and *trans*-coutaric acids, 2-*S*-glutathionylcaftaric acid, and oxidized cinnamic acids were quantitated as chlorogenic acid, while a hydroxybenzoic derivative was quantitated as protocatechuic acid and flavonols were quantitated as rutin. Concentration (C) of each individual phenolic in the samples was calculated from peak area (A) by using

$$C = \alpha + \beta A \quad (1)$$

where α is curve intercept and β is curve slope (for the corresponding compound).

Similar dilutions of a water solution of 80 mg/L catechin and 80 mg/L epicatechin and linear regression were used for the quantitation of catechins and procyanidins. All procyanidins were expressed as catechin. Equation 1 was modified to

$$C = [(\alpha + \beta A)/\mathrm{CF}](R/100) \quad (2)$$

to accommodate the concentration factor (CF) and the percent recovery (R) resulting from the isolation of these compounds with sample preparation. The average percent recovery, presented in Table I, of each individual procyanidin and catechin was used in eq 2.

Colorimetric Determination of Total Phenolics. Colorimetric determination of total phenolics was based on the procedure of Singleton and Rossi (1965). Grape juice (100 μL) filtered through a 0.45-μm Millipore (type HA) membrane was mixed with 900 μL of distilled water and 5 mL of 0.2 N Folin-Ciocalteau reagent (Sigma). Four milliliters of saturated sodium carbonate (75 g/L) was added to the mixture and then shaken. The absorbance of the solution at 765 nm was measured after 2 h with a Varian DMS 100 double-beam spectrophotometer. Quantitation was based on the standard curve of 100, 200, 300, 400, and 500 mg/L of gallic acid prepared at the same time.

Table I. Recoveries of Procyanidin Standard Solutions

soln[a]	replication	B3	B1	B4	catechin	B2	epicatechin
A	1	92.6	94.1	88.3	94.6	96.0	81.1
	2	93.6	92.1	93.3	93.4	96.1	81.9
	3	89.1	92.4	82.4	91.1	89.8	78.7
B	1	85.3	87.6	85.6	88.2	90.6	83.8
	2	95.4	89.5	91.6	91.6	92.1	77.1
	3	94.9	94.5	94.0	95.8	98.4	86.6
C	1	81.5	88.1	79.5	89.7	88.1	73.0
	2	81.5	92.3	80.1	92.7	90.6	85.6
	3	87.4	93.1	84.6	92.5	91.5	75.6
	4	84.8	89.9	79.3	87.7	89.9	70.1
min		81.5	87.6	79.3	87.7	88.1	70.1
max		95.4	94.5	94.0	95.8	98.4	86.6
av		88.6	91.4	85.9	91.7	92.3	79.4
SD		5.3	2.4	5.7	2.6	3.4	5.4
% CV		6.0	2.7	6.6	2.9	3.7	6.9

[a] Solution composition: (A) 43 mg/L of B3, 50 mg/L of B1, 34 mg/L of B4, 81 mg/L of B2, 110 mg/L of catechin, and 85 mg/L of epicatechin; (B) 17.2 mg/L of B3, 20 mg/L of B1, 13.6 mg/L of B4, 32.4 mg/L of B2, 44 mg/L of catechin, and 34 mg/L of epicatechin spiked with chlorogenic, caffeic, coumaric, and ferulic acids; (C) 5.8 mg/L of B3, 6.2 mg/L of B1, 4.2 mg/L of B4, 10.1 mg/L of B2, 13.7 mg/L of catechin, and 10.6 mg/L of epicatechin.

RESULTS AND DISCUSSION

Characterization of Phenolic Acids and Flavonols. A typical chromatogram of phenolic acids and flavonol glycosides from Thompson Seedless grape juice is shown in Figure 2. Identification of the trans isomers of caftaric, coutaric, caffeic, *p*-coumaric, and ferulic acids (peaks 8, 12, 13, 14, and 15, respectively) was based on comparisons of retention and spectral characteristics of the corresponding peaks with those of standards. Presence of gallic acid and rutin (peaks 3 and 17, respectively) was also verified by both retention and spectral data, while presence of another quercetin glycoside (peak 18) was

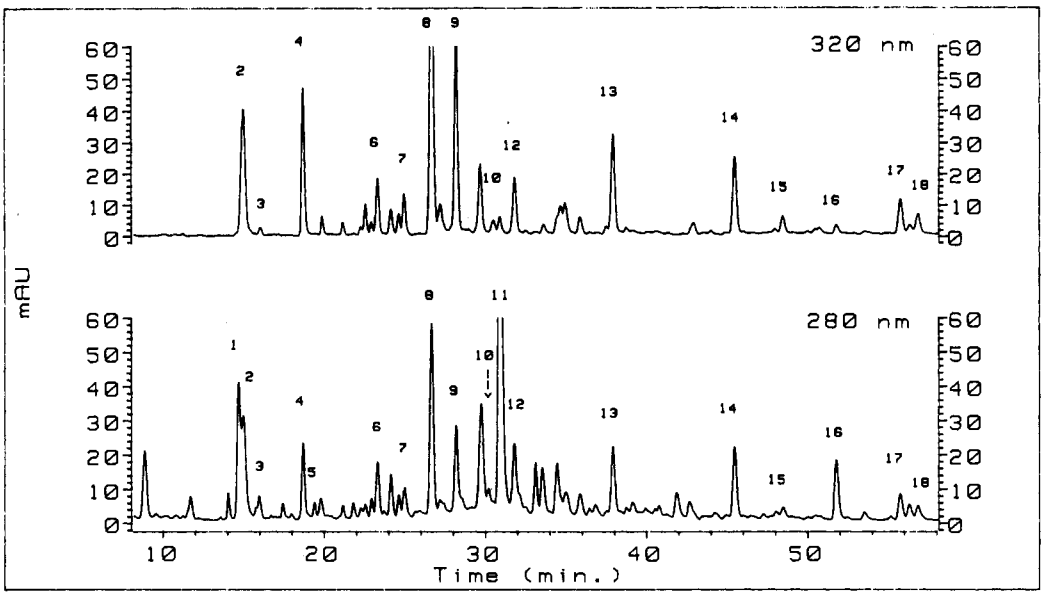

Figure 2. HPLC chromatogram of Thompson Seedless phenolic acids and flavonols. Peaks: (1) tyrosine; (2) oxidized cinnamic; (3) gallic acid; (4) oxidized cinnamic; (5) HMF; (6) oxidized cinnamic; (7) *cis*-caftaric; (8) caftaric; (9) glutathionylcaftaric; (10) *cis*-coutaric; (11) tryptophan; (12) *p*-coutaric; (13) caffeic; (14) *p*-coumaric; (15) ferulic; (16) hydroxybenzoic ester; (17) rutin; (18) quercetin glycoside.

clearly indicated by the spectral data. Peaks 7 and 10 were tentatively identified as *cis*-caftaric and *cis*-coutaric acids, respectively. Cis isomers of cinnamics are present in grapes (Singleton et al., 1978; Baranowski and Nagel, 1981; Jaworski and Lee, 1987), and they are eluted before the corresponding trans isomers in reverse-phase HPLC systems (Baranowski and Nagel, 1981; Jaworski and Lee, 1987). Transformation of *trans*- to *cis*-coutaric acid can occur under UV light (Singleton et al., 1978). When standards of caftaric and coutaric acids were treated with UV light, peaks with the same retention times and spectra as those of peaks 7 and 10 were generated. Peak 9, which is a major 320-nm peak in the phenolic profile of Thompson Seedless grape juice, was identified as 2-*S*-glutathionylcaftaric acid. It has been well established that oxidation of caftaric acid, catalyzed by polyphenol oxidase (PPO), leads to the formation of glutathionylcaftaric acid (Singleton et al., 1984; 1985; Cheynier et al., 1986; Cheynier and Van Hulst, 1988). The spectrum of peak 9, which is shown in Figure 3a, relates very well with the spectrum of 2-*S*-glutathionylcaftaric acid published by Cheynier et al. (1986). Peak 9 has a slightly longer retention than caftaric acid. Cheynier et al. (1986) also report that 2-*S*-glutathionylcaftaric acid elutes shortly after caftaric acid in a reverse-phase HPLC system that uses methanol as mobile phase.

In addition to phenolics, non-phenolic compounds such as amines and aminophenols are expected to contribute to the complex chromatographic pattern of grape juice (Singleton and Trousdale, 1983) as non-phenolic substances can have appreciable absorbance in the UV (Somers and Ziemelis, 1972; Myers and Singleton, 1979). L-Tyrosine (peak 1), tryptophan (peak 11), and HMF (peak 5) were non-phenolic compounds identified by matching both retention and spectral data of the corresponding peaks with those of standards. Tyrosine and tryptophan are known to occur in grape juices and wines (Kluba et al., 1978; Sanders and Ough, 1985). Although HMF was barely detected in the chromatogram of Figure 2, it corresponded to a significant peak in the chromatograms of bottled juices and stored concentrates. Peaks 2, 4, 6, and 16 were not completely identified. Some information about the nature of the aglycon of these compounds, however, can be obtained from the spectra of these peaks. The spectrum of peak 2 is identical with the spectrum of peak 6 and is shown in Figure 3b. The similarity in the spectral characteristics and the difference in retention times of peaks 2 and 6 indicate that these peaks have the same aglycon, but peak 2 is more polar than peak 6. The spectrum of peak 4, shown in Figure 3c, exhibits the general characteristics of cinnamic spectra such as those of caffeic or ferulic acids. Compounds having spectra identical with those of peaks 2 and 4 but longer retention times (less polar) were found and isolated from pear juice processed with SO_2 (Spanos and Wrolstad, 1990). The compounds were tentatively characterized as products of cinnamic oxidation formed in the presence of sulfites. Since grapes have considerable content of sulfhydryl compounds (Cheynier et al., 1986), these peaks may correspond to products of similar nature. Peak 16 exhibited a spectrum (Figure 3d) similar to that of hydroxybenzoic acids such as vanillic or protocatechuic acids, but it had a longer retention time than these compounds. It is likely that this peak corresponds to a hydroxybenzoic acid esterified with a hydrophobic moiety.

Influence of Processing and Storage on the Phenolic Acids and Flavonols. Table II shows the cinnamic acid composition of Thompson Seedless grape juice at the different processing and storage stages. The levels of gallic acid and quercetin glycosides along with levels of tyrosine, tryptophan, and HMF and the changes that occur with processing and storage are shown in Table III. The phenolic acid composition of grape juice processed with addition of SO_2 is consistently higher than that of grape juice processed without SO_2 addition. The levels of each cinnamic acid amount to less than 1 mg/L in the juice processed without SO_2. The presence of the reducing agent during processing results in less oxidation and, consequently, in higher phenolic acid concentrations. The levels of SO_2, however, are not high enough to exclude any oxidation. Enzymatic oxidation of caftaric acid to caftaric *o*-quinone by PPO can be followed by spontaneous addition of glutathione. The high content of glutathione in Thompson Seedless grapes allows for quantitative formation of 2-*S*-glutathionylcaftaric acid (Cheynier et al., 1986). Caffeic and coumaric acids are found in the form

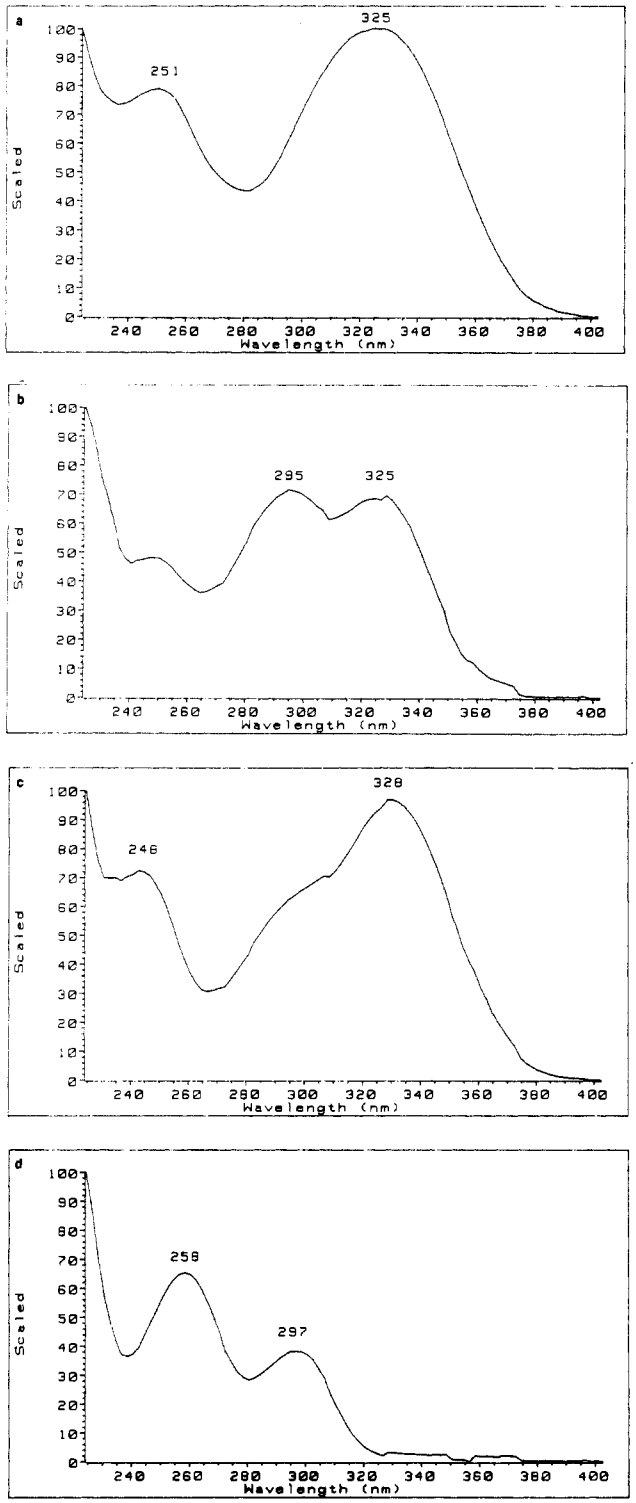

Figure 3. (a) UV spectrum of peak 9 (glutathionylcaftaric) (corresponding to Figure 2). (b) UV spectrum of peak 2 (corresponding to Figure 2). (c) UV spectrum of peak 4 (corresponding to Figure 2). (d) UV spectrum of peak 16 (corresponding to Figure 2).

of their tartrate esters in the SO_2-processed juice sampled at the press stage. Juice clarification, however, caused almost complete hydrolysis of the tartrate esters, and as a result the subsequent processing stages as well as the finished juices contain mostly the free forms of caffeic and coumaric acids. Esterase activity present in the enzymatic preparation used for clarification probably accounts for the observed hydrolysis. Similar hydrolytic activity on cinnamics by pectinase preparations have been reported (Baranowski and Nagel, 1981; Spanos and Wrolstad, 1990, Spanos et al., 1990). Hydrolysis of glutathionylcaftaric acid to glutathionylcaffeic acid during clarification was not observed. The data on the quercetin glycosides (Table III) show little effect of SO_2 on the oxidation of quercetin constituents. It has been reported that flavonol glycosides are not direct PPO substrates (Baruah and Swain, 1959). There is apparent hydrolysis of rutin during enzymatic hydrolysis. Storage of concentrates for 9 months at 25 °C resulted in extensive degradation of phenolic acids, total loss of quercetin glycosides, and formation of high amounts of HMF (up to 33.5 mg/L). Some HMF was also formed during the heat treatment of bottling. Hardly any HMF, however, was formed with the process of concentration. The effect of SO_2 on the concentration of peaks 2, 4, and 6 (Table II) is similar to that on the identified phenolic acids but there is no apparent hydrolytic activity on these peaks similar to that observed on the cinnamics. It is worth noting that drastic changes in the composition of tyrosine and tryptophan occurred with storage (Table III). Total loss of tryptophan was observed, while tyrosine was reduced by more than 50%. The involvement of these amino acids in browning reactions may account for their degradation (Wrolstad et al., 1989).

Characterization of Procyanidins. Figure 4a shows a chromatogram of procyanidin standards. The retention times show that procyanidins have similar retention to other phenolic compounds, especially cinnamic acids. The poor resolution of procyanidins from other interfering compounds and their spectral characteristics (280-nm absorbance and relatively low extinction coefficient) require selective removal of cinnamic acids and other interfering compounds during sample preparation. We based our initial efforts to isolate procyanidins from the phenolic acids on the ionization of phenolic acids at pH 7. However, ethyl acetate extraction of grape juice at pH 7 as described by Salagoîty-Auguste and Bertrand (1984) resulted in highly variable recoveries of procyanidins and in coextraction of interfering cinnamics. Absorption of phenolics on a C_{18} Sep-Pak cartridge, selective elution of phenolic acids with 5% methanol at pH 7, and subsequent elution of procyanidins with methanol gave similar poor results.

A typical chromatogram of procyanidins isolated from grape juice with Sephadex LH-20 is shown in Figure 4b. The spectral data and the 320-nm signal of the chromatogram indicate that the sample preparation effectively removes cinnamic acids that coelute with procyanidins (this is particularly important in commodities, such as apples and pears, that contain chlorogenic acid and isomers of chlorogenic acid). Caffeic acid, coumaric acid and quercetin derivatives that remain in the procyanidin fraction do not interfere with the elution of procyanidins. Table I shows the recoveries of catechin/procyanidin standard solutions subjected to the Sephadex LH-20 sample preparation procedure. The recoveries and the degree of reproducibility between preparations as indicated by the coefficient of variation (% CV) are high for every individual catechin/procyanidin. Recovery was not affected by procyanidin concentration over the range tested. Some epicatechin elutes from the Sephadex LH-20 when the column is washed with 20% methanol. This probably accounts for the slightly lower and more variable recovery of epicatechin.

Influence of Processing and Storage on the Procyanidins. Table IV shows the influence of processing and storage on the procyanidin composition of Thompson Seedless grape juice. B1 (dimer of epicatechin–catechin) is the major dimer with low levels of B2 (dimer of epicatechin), B3 (alternative stereochemistry of B1), and B4 (dimer of catechin) also present. The levels of cate-

Table II. Influence of Processing and Storage on the Cinnamic Composition (Milligrams per Liter) of Thompson Seedless Grape Juice

treatment	oxidized cinnamics[a] A	B	C	caftaric[b] cis	trans	2-S-glutathionylcaftaric[b]	coutaric[b] cis	trans	caffeic	coumaric	ferulic	total cinnamics[c]
+SO_2												
press	6.7	4.9	2.0	1.0	14.0	7.6	0.2	2.2	2.4	1.6	0.4	43.0
enzyme clarif	6.5	4.5	1.9	0.1	2.2	7.3	tr[d]	0.4	8.1	3.2	0.5	34.6
filtered, fined	5.1	3.8	1.9	0.0	1.7	6.6	tr	0.4	7.9	3.0	0.5	30.8
bottled, fined	5.3	3.6	1.8	tr	2.6	6.3	tr	0.3	7.6	3.0	0.5	31.0
concentrated, fined	5.4	3.0	1.8	0.0	1.1	5.9	0.0	0.3	8.5	3.1	0.5	29.7
concentrate, stored	2.0	0.0	0.0	0.0	0.0	3.5	0.0	0.0	4.9	2.7	0.1	13.3
filtered, not fined	3.5	3.5	1.6	0.0	1.3	6.7	0.0	0.3	8.4	3.3	0.5	29.1
bottled, not fined	4.8	3.4	1.8	0.3	2.6	6.0	0.0	0.3	7.4	3.1	0.5	30.2
concentrated, not fined	4.9	2.8	1.1	0.0	0.5	5.3	0.0	0.2	8.5	3.3	0.5	27.1
concentrate, stored	1.3	0.0	0.0	0.0	0.0	2.5	0.0	0.0	4.4	2.9	tr	11.1
-SO_2												
press	2.3	2.0	0.4	0.0	0.6	8.4	0.0	0.4	0.2	0.3	0.0	14.7
enzyme clarif	2.5	1.9	0.7	0.0	0.8	8.2	0.0	0.3	0.5	0.2	0.3	15.4
filtered, fined	1.2	1.2	0.4	0.0	0.9	8.2	0.0	0.2	0.9	0.9	0.2	14.3
bottled, fined	1.5	1.1	0.8	0.0	0.5	6.6	0.0	0.2	0.9	0.8	0.0	12.3
concentrated, fined	1.3	1.1	0.3	0.0	0.4	6.6	0.0	0.5	0.8	1.1	0.3	12.3
concentrate, stored	0.2	0.5	0.0	0.0	0.0	2.9	0.0	0.0	0.7	1.1	0.1	5.4
filtered, not fined	1.7	1.3	0.4	0.0	0.9	8.6	0.0	0.3	1.1	0.8	0.3	15.5
bottled, not fined	1.7	1.3	0.6	0.0	0.5	7.8	0.0	0.2	1.1	1.0	0.2	14.4
concentrated, not fined	1.5	1.3	0.6	0.0	0.4	7.1	0.0	0.2	1.3	1.4	0.2	13.9
concentrate, stored	0.1	0.4	0.0	0.0	0.0	4.0	0.0	0.0	1.0	1.5	0.1	7.2

[a] Compounds A, B, and C correspond to peaks 2, 4, and 6, respectively, of Figure 2, quantitated as chlorogenic acid. [b] Quantitated as chlorogenic acid. [c] Includes oxidized cinnamics. [d] Less than 0.2 mg/L.

Table III. Influence of Processing and Storage on the Hydroxybenzoic Acid, Flavonol Glycoside, HMF, Tyrosine, and Tryptophan Composition (Milligrams per Liter) of Thompson Seedless Grape Juice

treatment	gallic	hydroxybenzoic deriv[a]	rutin	quercetin glycoside[b]	HMF	tyrosine	tryptophan
+SO_2							
press	1.2	4.1	5.5	3.6	0.0	33.2	39.4
enzyme clarif	1.4	7.0	tr[c]	4.5	0.8	34.9	39.0
filtered, fined	1.5	6.5	tr	4.5	0.7	32.3	39.7
bottled, fined	1.6	2.6	tr	7.5	2.3	30.1	33.4
concentrated, fined	1.6	6.3	tr	2.6	0.5	29.2	33.7
concentrate, stored	0.0	0.0	0.0	0.0	32.2	14.9	0.0
filtered, not fined	1.4	6.1	0.0	3.3	0.8	33.8	39.5
bottled, not fined	1.8	1.2	tr	7.2	5.4	31.2	35.4
concentrated, not fined	1.6	5.8	0.0	2.1	0.5	30.1	34.5
concentrate, stored	0.0	0.0	0.0	0.0	31.1	13.8	0.0
-SO_2							
press	0.7	2.4	5.7	3.5	0.0	32.8	37.7
enzyme clarif	0.6	2.5	2.2	4.3	0.0	31.9	39.0
filtered, fined	0.8	3.3	1.8	3.5	0.0	31.5	36.8
bottled, fined	0.8	0.0	1.4	6.3	7.1	30.3	24.8
concentrated, fined	0.9	4.1	tr	1.4	0.0	29.7	33.1
concentrate, stored	0.0	0.0	0.0	0.0	28.9	16.2	0.0
filtered, not fined	0.8	2.2	1.7	4.2	0.0	32.2	39.0
bottled, not fined	0.5	0.0	1.5	7.5	2.7	31.8	29.2
concentrated, not fined	0.7	3.6	tr	1.7	0.0	29.1	31.6
concentrate, stored	0.0	0.0	0.0	0.0	33.5	12.9	0.0

[a] Quantitated as protocatechuic acid. [b] Quantitated as rutin. [c] Less than 0.8 mg/L.

chin exceed the levels of epicatechin. The trimer and tetramer are partially resolved, and they are quantitated as the total. These procyanidin structures as well as catechin and epicatechin have been previously reported to be present in wines (Lea et al., 1979; Lee and Jaworski, 1987). A number of peaks (up to eight) showed typical catechin procyanidin spectra, and they were assigned as unknown procyanidins. The data (Table IV) show that the effect of SO_2 on the concentration of procyanidins is similar to that on phenolic acids previously discussed. Grapes processed without SO_2 contain about half of the procyanidins of the SO_2-processed grapes. The higher procyanidin content of the SO_2-processed grapes is apparently due to the inhibition of oxidation of procyanidins by the reducing agent. Fining resulted in no apparent changes of the procyanidin composition, but it appears that procyanidin degradation occurred with bottling and concentration. It is known that procyanidins become very sensitive to nonenzymatic condensation and polymerization at elevated temperature. Degradation during concentration was more severe than during bottling in all cases except that of the fined grape juice processed without SO_2. In this bottled juice, however, the levels of HMF were the highest of all the bottled juices, which indicates that the sample received more heat treatment during bottling. Complete loss of procyanidins occurred during storage of concentrates for 9 months at 25 °C.

Total Phenolics by HPLC and by the Colorimetric Assay. Table V shows the total phenolic composition of grape juice as determined by HPLC and by the colo-

Table IV. Influence of Processing and Storage on the Procyanidin Composition[a] (Milligrams per Liter) of Thompson Seedless Grape Juice

	B3	B1	B4	catechin	B2	trimer + tetramer	epicatechin	total unknowns[b]	total procyanidins[c]
$+SO_2$									
press	3.4	13.9	2.6	6.6	1.3	1.0	2.0	10.4	41.3
enzyme clarif	4.7	15.4	3.5	7.4	1.8	1.5	2.1	12.5	49.0
filtered, fined	4.1	14.2	3.2	6.7	1.2	1.9	1.9	12.2	45.5
bottled, fined	3.7	11.4	2.7	6.3	1.1	1.0	1.8	8.7	36.7
concentrated, fined	2.8	7.7	2.2	5.2	1.0	0.9	1.3	6.8	27.9
concentrate, stored	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
filtered, not fined	4.4	15.0	3.5	7.0	1.3	2.3	2.2	14.1	49.9
bottled, not fined	3.8	12.1	3.2	6.5	1.2	1.4	1.9	10.0	40.3
concentrated, not fined	2.2	6.2	1.8	4.9	0.8	1.2	1.3	6.7	25.2
concentrate, stored	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
$-SO_2$									
press	2.4	7.9	1.8	2.9	0.8	0.9	0.8	8.0	25.4
enzyme clarif	2.9	7.7	1.9	2.2	1.1	1.0	1.1	8.1	25.9
filtered, fined	2.7	7.9	2.8	3.0	0.8	0.6	1.0	7.4	26.2
bottled, fined	0.7	1.9	tr[d]	1.7	tr	tr	tr	1.0	5.5
concentrated, fined	0.6	2.3	0.6	1.6	0.0	0.0	tr	1.1	6.4
concentrate, stored	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
filtered, not fined	2.9	8.1	2.1	3.1	1.2	0.4	1.1	7.4	26.3
bottled, not fined	1.5	4.3	1.2	2.7	tr	tr	0.6	3.8	14.1
concentrated, not fined	0.9	3.1	0.6	1.8	0.2	tr	0.3	2.3	9.2
concentrate, stored	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a] Procyanidins B1, B2, B3, B4, trimer, tetramer, and total unknowns are quantitated as catechin. [b] Marked with "×" on Figure 4b. [c] Includes catechin and epicatechin. [d] Less than 0.2 mg/L.

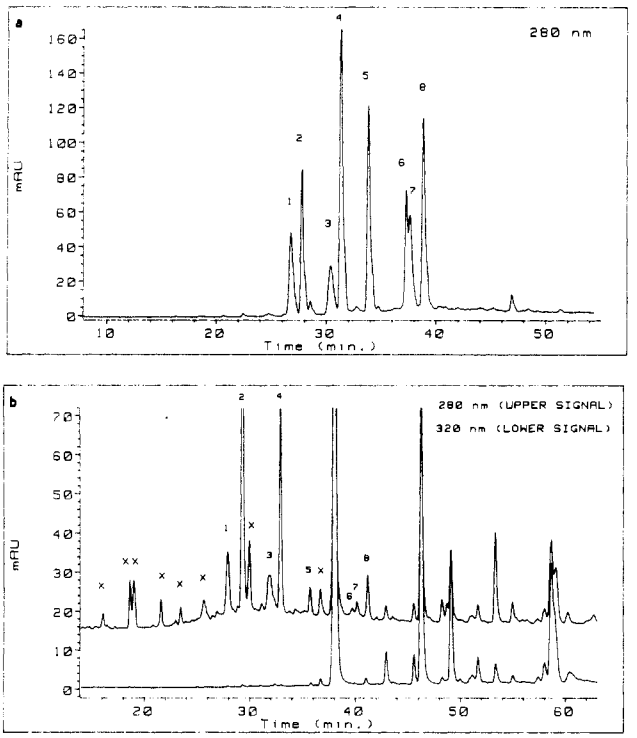

Figure 4. (a) HPLC chromatogram of procyanidin standards. Peaks: (1) procyanidin B3; (2) procyanidin B1; (3) procyanidin B4; (4) catechin; (5) procyanidin B2; (6) trimer; (7) tetramer; (8) epicatechin. (b) HPLC chromatogram of Thompson Seedless grape procyanidins. "×" unknown procyanidins.

rimetric assay. The two methods show no correlation at all ($r^2 = 0.141$). Storage of concentrates resulted in decrease of phenolic levels as measured by HPLC. The colorimetric assay, however, showed increased levels of phenolics. According to the colorimetric assay, juice sampled at the clarification stage is higher in phenolics than juice sampled at the press stage. If the clarified juice is fined, then the levels of phenolics are reduced to the levels found at the press stage. If the juice, however, is subsequently processed without fining, then the levels of phenolics remain high. HPLC data do not demonstrate

Table V. Total Phenolic by HPLC and by the Colorimetric Assay

treatment	total phenolic by HPLC,[a] mg/L	total phenolic by colorimetric assay in mg/L of gallic acid
$+SO_2$		
press	98.6	379
enzyme clarif	96.5	426
filtered, fined	88.8	371
bottled, fined	79.5	365
concentrated, fined	68.0	369
concentrate, stored	13.3	478
filtered, not fined	89.7	421
bottled, not fined	80.7	408
concentrated, not fined	61.8	393
concentrate, stored	11.1	492
$-SO_2$		
press	52.4	317
enzyme clarif	50.9	361
filtered, fined	49.9	320
bottled, fined	26.3	315
concentrated, fined	25.1	312
concentrate, stored	5.4	369
filtered, not fined	50.7	378
bottled, not fined	38.0	352
concentrated, not fined	29.0	353
concentrate, stored	7.2	391

[a] Sum of cinnamics, hydroxybenzoics, flavonols, and procyanidins.

such changes. When the preparation used for clarification was subjected to the colorimetric assay for total phenolics, no readings were obtained. These observations suggest that some compounds which are responsive to the colorimetric assay are formed during clarification but are removed with fining. Somers and Ziemelis (1980) reported synergistic interference between SO_2 and *o*-dihydroxyphenol which becomes very significant as the ratio of SO_2 to total phenolics increases. Our data do not indicate such a relation at least for the SO_2 levels (ca. 70 ppm) used in our study. It seems that products of phenolic degradation and nonenzymatic browning intermediates such as ene-

diols and reductones are the major reasons for the lack of correlation between these methods.

CONCLUSION

The utility of a reverse-phase HPLC system coupled with diode array detection for characterization of phenolics in grape juice was demonstrated. Effective fractionation of procyanidins based on Sephadex LH-20 chromatography was also described.

The phenolic profile of Thompson Seedless grape juice showed profound quantitative differences between the grapes processed with and without SO_2 as both phenolic acids and procyanidins were present in much lower levels in the juice processed without SO_2. Quercetin derivatives, however, were not affected by the presence of SO_2. The commercial enzyme preparation used for clarification showed hydrolytic activity on caftaric, coutaric, and quercetin glycosides. Procyanidins were shown to be sensitive to degradation by the heat applied with bottling or concentration. Drastic degradation of phenolics and formation of HMF occurred during storage. The lack of correlation between HPLC and colorimetric quantitation of total phenols is due to specificity differences between the two methods.

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