Influence of Processing and Storage on the Phenolic Composition of Apple Juice†

George A. Spanos, Ronald E. Wrolstad,* and David A. Heatherbell

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

HPLC techniques were used to study the effect of processing, concentration, and storage on the phenolic composition of Granny Smith apple juice pressed from fruit held at 1 °C for 3 and 9 months. Extensive phenolic degradation was prevented by initial high-temperature short-time (HTST) treatment. Enzymatic clarification caused cinnamic hydrolysis and some procyanidin degradation. Fining and concentration had little effect on phenolics. Storage of concentrates for 9 months at 25 °C resulted in (hydroxymethyl)-furfural (HMF) formation (up to 27.9 mg/L), degradation of cinnamics (ca. 36%) and quercetin and phloretin glycosides (ca. 60%), and total loss of procyanidins. The effect of diffusion extraction at 55, 63, 67, and 73 °C on the phenolic composition of Red Delicious, McIntosh, and Spartan apple juice was also studied. Up to a 3-fold increase in cinnamics and a 5-fold increase in phloretin glycosides was measured in diffusion-extracted juices relative to pressed juices. The effect of diffusion extraction on increased levels of procyanidins and phloretin and quercetin derivatives was even more pronounced.

INTRODUCTION

The phenolic composition of apple juice consists of cinnamic acids such as esters of caffeic and coumaric acid with quinic acid (Van Buren, 1976; Lea, 1984), flavonols such as quercetin glucoside (Dick et al., 1987; Oleszek et al., 1988), dihydrochalcones such as phloridzin (Whiting and Coggins, 1975; Wilson, 1981; Oleszek et al., 1988), catechins such as epicatechin, and procyanidins such as dimer B2 (Lea and Timberlake, 1974; Lea, 1978).

The important role of phenolics, in taste characteristics, such as bitterness and astringency, formation of yellow and brown pigments, and hazes and sediments, has been realized (Lea and Arnold, 1978; Heatherbell, 1984; Lea, 1984). Although it has been reported (Van Buren, 1976; Lea and Timberlake, 1978) that variety, processing techniques, and storage conditions of finished products influence the phenolic composition of apple juice, most of the reports are based on semiquantitative methods such as TLC or colorimetric procedures. New technologies such as diffusion extraction and belt pressing that are being increasingly adopted by industry because of increased juice yields are expected to affect the phenolic composition of the juices (Schobinger et al., 1978). Recent advances in analytical techniques, especially in diode array detection, allow for more accurate characterization of the phenolic profile and the changes that occur during processing and storage.

This paper describes the characterization of the phenolic profile of apple juices with the use of diode array detection. The changes that occur with pasteurization, enzymatic clarification, fining, bottling, concentration, and storage on the phenolic composition of Granny Smith apple juice are investigated. The effect of diffusion extraction at different temperatures compared to conventional pressing on the phenolic composition of apple juice from Red Delicious, McIntosh, and Spartan fruit is also quantitated.

* To whom correspondence should be addressed.

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MATERIALS AND METHODS

Standards. Phenolic standards (chlorogenic, caffeic, and *p*-coumaric acids, phloridzin, rutin, catechin, and epicatechin) and (hydroxymethyl)furfural (HMF) were obtained from Sigma Chemical Co. Procyanidin standards (B1, B2, B3, B4, trimer, and tetramer) were provided by Dr. A. G. H. Lea of Cadbury Schweppes Ltd., Lord Zuckerman Research Center, University of Whiteknights, Reading, U.K. All the solvents used were of HPLC grade.

Samples. *Preparation of Granny Smith Apple Juice.* Granny Smith apples obtained in Fall 1986 from the Mid-Columbia Experiment Station, Hood River, OR, were held in storage at 1 °C for 3 and 9 months and then pressed into juice. The processing of the short- and long-stored apples was performed at the pilot plant of Oregon State University, Department of Food Science, according to the flow diagram shown in Figure 1. Samples were obtained in the intermediate processing stages (marked on Figure 1) and stored at −30 °C until needed. Concentrates, which had been kept frozen or stored for 9 months at 25 °C, were diluted to the original degrees Brix before analysis. More details about the processing are described elsewhere (Wrolstad et al., 1989).

Diffusion-Extracted Apple Juice. Apple juice samples from Red Delicious, McIntosh, and Spartan apples prepared at Agriculture Canada, Food Processing Section, Summerland, BC, with both conventional pressing techniques (rack and cloth press) and diffusion extraction at 55, 63, 67, and 73 °C were provided by Dr. D. B. Cumming and Dr. T. H. J. Beveridge. These samples were received and kept as single-strength frozen juice. Detailed information about the processing history of these samples is described by Cumming (1986).

Commercial Apple Juice Samples. Four commercial apple juice concentrates of European origin (A–D) were supplied by Pepsico Inc. (New York). Concentrates C and D had been decolorized by treatment with activated carbon and then reconcentrated. Samples A and B had not undergone reprocessing with activated carbon.

Determination of Apple Juice Phenolics with HPLC. The methodology and equipment for sample preparation, separation, and quantitation of phenolics in grape juice previously described by Spanos and Wrolstad (1990) was used. *p*-Coumarylquinic acid was quantitated as chlorogenic acid, phloretin xyloglucoside was quantitated as phloridzin, and flavonol glycosides were quantitated as rutin. Procyanidin quantitation was based on the standard curve of catechin. Standards were run intermittently throughout the day. All HPLC analyses were replicated, the mean values being reported; reproducibility was ca. ±5%.

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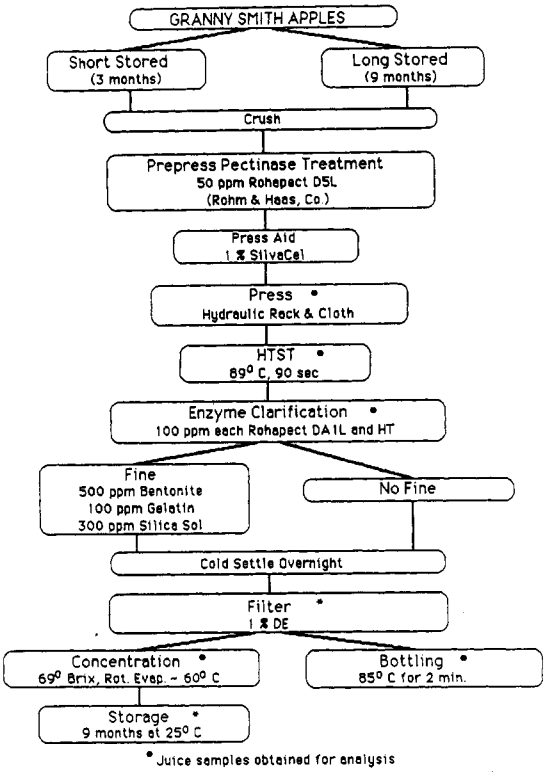

Figure 1. Outline of steps utilized in processing of Granny Smith apple juice.

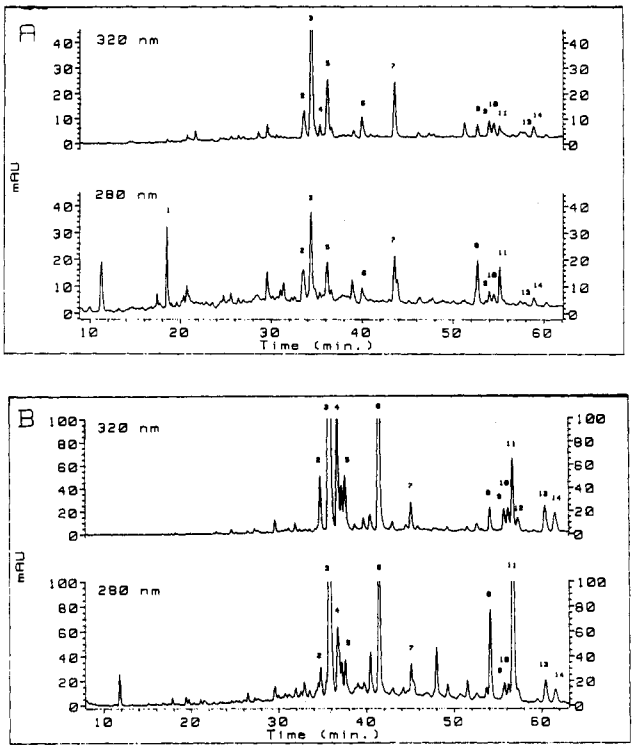

Figure 2. HPLC chromatogram of cinnamics, dihydrochalcones, and flavonols. (A) Granny Smith (extracted with conventional pressing); (B) McIntosh (diffusion extracted at 67 °C). Peaks: (1) HMF; (2) conjugated coumaric; (3) chlorogenic; (4) chlorogenic isomer; (5) caffeic; (6) *p*-coumarylquinic; (7) *p*-coumaric; (8) phloretin xyloglucoside; (9) quercetin galactoside; (10) quercetin glucoside; (11) phloridzin; (12) quercetin xyloside; (13) quercetin arabinoside; (14) quercetin rhamnoside.

Dilutions (1:0, 1:2, 1:4, and 1:6) of an aqueous solution containing the following compounds were used to prepare a standard curve: chlorogenic acid (80 mg/L), caffeic acid (25 mg/L), *p*-coumaric acid (10 mg/L), rutin (12 mg/L), phloridzin (20 mg/L), and HMF (20 mg/L). For the quantitation of catechins and procyanidins, the same dilutions for an aqueous solution of catechin (80 mg/L) and epicatechin (80 mg/L) were used.

Isolation and Hydrolysis of Chromatographic Peaks. Apple juice (30 mL) was adjusted to pH 2 with H_2SO_4 and extracted three times with 60, 30, and 30 mL of ethyl acetate. The extracts were combined, evaporated to dryness on a rotary evaporator, and dissolved in 2 mL of water.

Individual peaks from multiple injections of the isolate on the HPLC system were collected with a Gilson FC 203 fraction collector as they were eluted from the analytical column. These peaks were concentrated by absorption on a C_{18} Sep-Pak, elution with methanol, and evaporation of methanol on a rotary evaporator. One milliliter of peak isolates (ca. 600 mg/L) was subjected to alkaline hydrolysis for 10 h with 0.5 mL of 10% KOH under nitrogen at room temperature in the dark. The hydrolysates were acidified with 2 N HCl, and the aglycons were identified by the HPLC system for analysis of phenolics.

For identification of quinic acid, the hydrolysate was adjusted to pH 6–7 with NH_4OH and applied to an anion-exchange resin (1-mL column bed of Bio-Rex 5, Bio-Rad Laboratories). The bed was eluted with 0.5 mL of 10% H_2SO_4 followed by 3 mL of water, and the eluate was freeze-dried to ca. 0.2 mL. Quinic acid was detected by the reverse-phase HPLC system for analysis of nonvolatile acids in fruit juice as described by Coppola and Starr (1986).

Colorimetric Determination of Total Phenolics. Total phenolics were determined with the colorimetric procedure of Singleton and Rossi (1965) as described previously by Spanos and Wrolstad (1990).

RESULTS AND DISCUSSION

Characterization of Apple Phenolics. An HPLC chromatogram of cinnamics and phloretin and quercetin glycosides of juice processed with conventional pressing from Granny Smith apples is shown in Figure 2A. A similar chromatogram of juice diffusion extracted at 67 °C from McIntosh apples is shown in Figure 2B. Peak assignment was based on the retention time of standards, spectral data of the peaks, and in some cases on peak isolation and hydrolysis. Spectra were taken at the leading edge, the apex, and the tailing edge to monitor for peak purity as well as to characterize the aglycon. Although esterification of the aglycon with an acid or a sugar moiety may have little effect on the UV characteristics, it has significant effect on the polarity of the molecule. Consequently, the retention time of the peak may give tentative information about the nature of the esterification. Peak 2 was identified as a derivative of coumaric acid. The spectral data of the peak were very closely related to those of coumaric acid. In addition, peak isolation and hydrolysis yielded coumaric acid. Peak 4 exhibited spectral characteristics identical with those of chlorogenic acid, and it was tentatively identified as an isomer of chlorogenic acid. Positional isomers of chlorogenic acid such as neochlorogenic acid have been reported in apples (Van Buren, 1970), and they can be separated by HPLC (Court, 1977). Geometrical isomers (cis-) of cinnamics are also possible (Singleton et al., 1978). Peak 6 was identified as coumarylquinic acid. Spectral data indicated that the aglycon is coumaric acid, and this was verified by isolation and hydrolysis of the peak. Quinic acid was also identified as a hydrolysis product of this peak. The retention data indicate that esterification of caffeic or coumaric acid with quinic acid results in earlier elution of the cinnamic by approximately 2 min. The alkaline hydrolyses performed were complete, but the yields based on the aglycons were about 15% due to the cinnamic degradation occurring in alkaline conditions.

Peaks 9, 10, and 12–14 exhibited typical quercetin

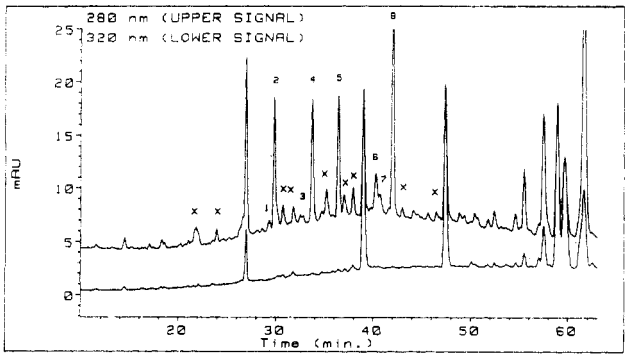

Figure 3. HPLC chromatogram of apple juice procyanidins. Peaks: (1) procyanidin B3; (2) procyanidin B1; (3) procyanidin B4; (4) catechin; (5) procyanidin B2; (6) trimer; (7) tetramer; (8) epicatechin; (×) unknown procyanidins.

spectra, and they were tentatively identified as the 3-*O*-galactoside (gal), 3-*O*-glucoside (glu), 3-*O*-xyloside (xyl), 3-*O*-arabinoside (arab), and 3-*O*-rhamnoside (rhamn) glycosides of quercetin. Oleszek et al. (1988) identified the presence of these five quercetin glycosides in apple skins by means of mass spectroscopy, and they report that the elution order of the quercetin glycosides on reverse-phase C_{18} HPLC system is galactoside, glucoside, xyloside, arabinoside, rhamnoside. The HPLC system used in this study was very similar to that of Oleszek et al. (1988), allowing for tentative assignment of peak identity. Peak 8 was identified as phloretin xyloglucoside (xylglu). The spectrum indicated that the aglycon is phloretin, and the retention time indicated a more polar compound than phloridzin (phloretin glucoside). Phloretin xyloglucoside has been reported as a phloretin derivative other than phloridzin present in apples (Whiting and Coggins, 1975; Wilson, 1981; Oleszek et al., 1988).

An HPLC chromatogram of procyanidins isolated with Sephadex LH-20 from apple juice is shown in Figure 3. Peaks marked with × on the chromatogram showed typical catechin spectra, and they were assigned as unknown procyanidins. Isolation of procyanidins with sample preparation and analysis in a separate run are necessary because they are eluted at similar retention times with phenolic acids and detected at 280 nm (Lea, 1982; Spanos and Wrolstad, 1990). The recoveries of procyanidins and the reproducibility of sample preparation procedure have been previously reported (Spanos and Wrolstad, 1990).

Influence of Processing and Storage on the Cinnamics. Table I shows the cinnamic and HMF composition at the different processing and storage stages of juice extracted with conventional pressing from Granny Smith apples. Table II presents the effect of processing, pressing vs diffusion extraction at different temperatures, on the cinnamic composition of Red Delicious, McIntosh, and Spartan apple juice. Although chlorogenic acid is the major cinnamic in all varieties, the highest concentration occurs in Red Delicious and the lowest in Granny Smith. This is about a 6-fold concentration difference when the finished juices of the Granny Smith are compared with the juice from conventional pressing of Red Delicious fruit. Chlorogenic concentration in Spartan is higher than in McIntosh. The literature reports a wide range of levels of chlorogenic acid. Brause and Raterman (1982) reported concentrations of chlorogenic between 93 and 232 μg/g for laboratory-produced apple juices and between 0 and 208 μg/g for commercial apple juice. Lee and Wrolstad (1988) found levels of chlorogenic acid between 1.5 and 228 mg/L in Golden Delicious, Jonathan, McIntosh, and Granny Smith apple juices from different geographic origins. Coumarylquinic acid is present in significant amounts in Red Delicious, McIntosh, and Spartan apple juices and in minor amounts in Granny Smith apple juice. Whiting and Coggins (1975) found levels of coumarylquinic acid between 0.003 and 0.0769% w/v in ciders from different varieties, while Van Buren et al. (1976) reported the qualitative presence of coumarylquinic acid in Golden Delicious apple juice. Low levels of free caffeic and coumaric acids are present in all varieties.

There is no apparent difference in the cinnamic composition of Granny Smith apple juice pressed from fruit held in short-term vs long-term storage, but there are some profound changes in the cinnamic composition occurring with processing (Table I). Initial HTST treatment protected cinnamics from enzymatic oxidation during processing. The lowest levels of cinnamics are found in juice sampled at the press stage. It is known that polyphenol oxidase (PPO) activity takes place in the pulp before and during pressing and continues in the juice until HTST treatment (Van Buren et al., 1976; Lea and Timberlake, 1978; Lea, 1984). Juice sampled at the press stage did not get the heat treatment that would inactivate the enzyme, and the degradative activity on the cinnamics continued. HTST protects the cinnamics extracted in the juice from further PPO action. Clarification enzymes caused hydrolysis of conjugated cinnamics. The compositional data at the clarification stage show decreased levels of chlorogenic, conjugated coumaric, and coumarylquinic acids and increased levels of caffeic and coumaric acids relative to the compositional data at the HTST stage. The levels of chlorogenic acid are reduced by 8.7 mg/L, and the levels of caffeic acid increase by 3.7 mg/L. From the standard curves of these compounds it is expected that for every milligram of chlorogenic acid hydrolyzed approximately 0.5 mg of caffeic acid should be generated. The above effect of clarification enzymes is not apparent in the fined samples or in the finished bottled juices. It was found by use of electrophoresis techniques (Hsu et al., 1989) that pasteurization at the bottling stage precipitated enzymes added for clarification. They also found that concentration of the juice did not change its protein profile. Cinnamic hydrolysis is very evident in the filtered juice that was not fined and in the concentrate made from unfined juice. These data indicate that the hydrolytic activity continued after clarification, but was removed with fining and/or bottling. Storage of concentrates for 9 months at 25 °C resulted in HMF formation and approximate 36% loss in cinnamics. Increased levels of HMF and low levels of cinnamics may be a useful index of the storage history of apple concentrates.

Table II documents that diffusion extraction yields higher levels of cinnamics than conventional pressing. The increase of cinnamic concentration becomes more evident as the temperature of extraction increases. At elevated temperatures, cell membrane permeability increases, the concentration of oxygen decreases (because of lower solubility), and the enzymes become inactivated. These factors all account for the higher cinnamic yields (Cumming, 1986). Diffusion extraction at 55 °C results in a 3-fold increase in the cinnamic concentration of McIntosh and a 1.5-fold increase in Spartan juice, relative to the juice extracted with rack and cloth press. The cinnamic concentration, however, of the Red Delicious juice diffusion extracted at 55 °C is lower than that of pressed juice. When the diffusion extraction is performed at a relatively low temperature, any temperature drop (temperature fluctuations are not unusual in pilot plant

Table I. Influence of Processing and Storage on the Cinnamic and HMF Composition (Milligrams per Liter) of Granny Smith Apple Juice

treatment	HMF	chlorogenic	chlorogenic isomer[a]	caffeic	conjugated coumaric[a]	coumaryl-quinic[a]	coumaric	total cinnamics
short-stored fruit								
press	0.0	3.4	0.3	2.5	0.9	0.0	1.4	8.5
HTST	0.0	13.4	0.6	1.3	4.5	1.9	0.3	22.0
enzyme clarif	0.0	4.7	0.3	5.0	1.2	0.0	2.6	13.8
filtered, fined	0.0	9.5	0.5	2.7	1.9	1.0	1.6	17.2
bottled, fined	0.3	9.5	0.5	2.2	1.8	1.1	1.5	16.7
concentrated, fined	0.0	11.1	0.5	3.0	2.1	1.1	2.6	20.5
concentrate, stored	25.3	6.8	0.2	1.9	1.4	0.8	1.9	13.0
filtered, not fined	0.0	5.4	0.5	4.7	1.0	0.3	2.5	14.5
bottled, not fined	0.6	8.8	0.6	2.5	1.8	1.0	1.9	16.6
concentrated, not fined	0.0	6.4	0.4	4.0	0.9	0.5	2.4	14.6
concentrate, stored	27.9	2.3	0.6	2.6	0.6	0.9	1.9	8.9
long-stored fruit								
press	0.0	2.9	0.0	0.7	2.3	0.4	0.6	6.9
HTST	0.0	13.1	0.5	1.7	5.4	1.5	0.1	22.2
enzyme clarif	0.0	7.0	0.4	3.1	1.1	0.3	2.3	14.2
bottled, fined	0.0	9.5	0.5	1.9	2.2	1.0	1.8	16.9
concentrated, fined	0.0	12.1	0.7	2.6	3.1	1.1	2.4	22.0
bottled, not fined	0.0	9.7	0.6	3.2	2.2	1.0	3.2	19.9
concentrated, not fined	0.0	5.4	0.8	3.3	2.0	1.1	2.4	15.0

[a] Quantitated as chlorogenic acid.

Table II. Influence of Extraction Procedure (Pressing vs Diffusion Extraction) on the Cinnamic and HMF Composition[a] (Milligrams per Liter) of Apple Juice

treatment	HMF	chlorogenic	chlorogenic isomer[b]	caffeic	conjugated coumaric[b]	coumaryl-quinic[b]	coumaric	total cinnamics
Red Delicious								
pressed (control)	0.0	59.2	3.7	3.5	2.2	21.5	1.5	91.6
diffusion								
55 °C	0.0	27.5	3.5	6.4	0.0	10.8	5.7	53.9
63 °C	0.0	59.0	4.2	8.5	0.0	17.4	5.7	94.7
67 °C	0.0	67.8	4.0	8.0	0.0	19.7	5.3	104.7
73 °C	0.0	82.6	3.3	9.6	0.0	19.2	6.2	120.9
McIntosh								
pressed (control)	0.0	17.4	8.8	2.7	4.8	15.7	0.8	50.2
diffusion								
55 °C	0.0	82.5	14.3	5.8	5.7	31.6	1.3	141.2
67 °C	0.0	98.6	14.5	6.8	6.2	34.0	1.7	161.8
Spartan								
pressed (control)	0.0	34.7	2.9	1.9	7.1	10.9	0.7	58.4
diffusion								
55 °C	0.0	43.9	2.8	2.0	5.3	10.1	1.1	65.1
63 °C	0.0	98.8	3.9	3.5	8.0	14.5	1.0	129.8
73 °C	0.0	113.7	2.8	4.7	8.7	15.2	1.3	146.4

[a] Data normalized to 11.5° Brix. [b] Quantitated as chlorogenic acid.

scale operation) may facilitate oxidation. The cinnamic composition of the juice diffusion extraction at 73 °C is higher than that of the pressed juice by approximately 1.5 times for the Red Delicious, 3 times for the McIntosh, and 2.5 times for the Spartan juice.

Influence of Processing and Storage on the Quercetin and Phloretin Glycosides. Table III shows the quercetin and phloretin glycoside composition of Granny Smith apple juice. The quercetin and phloretin glycoside composition of Red Delicious, McIntosh, and Spartan apple juices is shown in Table IV. Up to four different quercetin glycosides were found in Granny Smith juice, up to five quercetin glycosides were quantitated in diffusion-extracted Red Delicious, McIntosh, and Spartan apple juices, but hardly any quercetin glycoside was detected in these juices when processed with conventional pressing. Walker (1964) reported that flavonol glycosides are concentrated in the epidermal tissues. Dick et al. (1987) identified quercetin glycosides (arabinoside, galactoside, glucoside, rhamnoside, and xyloside) in the peels of Spartan apples, and Olezek et al. 1988) found the same compounds in Rhode Island Greening apple skins. Durkee and Poapst (1965) found high levels of phloridzin in the core tissue and apple seeds. We found about 20 mg of phloridzin/g of seeds when we analyzed water extracts of seeds extracted overnight at room temperature. The presence of phloretin xyloglucoside in apple juice has been reported by Whiting and Coggins (1975), Wilson (1981), and Oleszek et al. (1988).

The lowest levels of quercetin and phloretin glycosides (Table III) were in the juice sampled at the press stage. Although flavanol glycosides are not direct substrates for PPO (Van Buren et al., 1976), it seems that HTST treatment has a protective effect against oxidation of these constituents similar to that observed for cinnamics. Clarification, fining, bottling, and concentration had no apparent effect on the quercetin and phloretin glycoside composition. Storage of concentrates at 25 °C for 9 months resulted in 50–60% loss of the quercetin and phloretin derivatives.

Diffusion extraction resulted in an extreme increase of quercetin and phloretin constituents (Table IV). This is

Table III. Influence of Processing and Storage on the Quercetin and Phloretin Glycoside Composition (Milligrams per Liter) of Granny Smith Apple Juice

treatment	quercetin 3-O-[a] gal	glu	xyl	arab	rhamn	total quercetin glycosides	phloretin[b] xylglu	phloridzin	total phloretin glycosides
short-stored fruit									
press	2.7	0.0	0.0	0.0	3.2	6.0	2.2	2.6	4.8
HTST	3.6	3.1	0.0	2.1	2.9	11.7	4.3	4.4	8.7
enzyme clarif	3.0	2.8	0.0	1.6	3.7	11.1	4.2	4.1	8.3
filtered, fined	3.1	2.6	0.0	2.9	1.0	9.7	4.2	4.3	8.5
bottled, fined	2.8	2.8	0.0	2.6	0.0	8.2	4.4	3.6	8.0
concentrated, fined	2.9	2.4	0.0	2.0	1.4	8.7	4.2	3.7	7.8
concentrate, stored	1.2	1.1	0.0	1.7	0.0	4.0	2.9	2.5	5.4
filtered, not fined	3.2	3.0	0.0	2.6	0.9	9.8	4.5	4.3	8.9
bottled, not fined	3.2	2.8	0.0	2.9	0.0	8.9	4.6	3.1	7.6
concentrated, not fined	3.9	3.2	0.0	2.0	1.6	10.7	5.6	3.6	9.1
concentrate, stored	1.0	0.9	0.0	0.0	2.4	4.3	2.3	1.7	4.0
long-stored fruit									
press	2.2	1.4	0.0	0.0	0.0	3.6	0.0	1.3	1.3
HTST	2.9	2.2	0.0	3.8	1.8	10.7	2.9	3.3	6.2
enzyme clarif	2.6	2.5	0.0	3.4	1.6	10.0	2.7	2.7	5.4
bottled, fined	2.1	1.8	0.0	2.4	0.0	6.3	2.2	2.3	4.6
concentrated, fined	2.8	2.3	0.0	3.1	0.0	8.2	2.8	2.8	5.6
bottled, not fined	3.2	2.4	0.0	3.1	0.0	8.8	2.9	2.7	5.6
concentrated, not fined	2.8	2.3	0.0	1.5	3.2	9.7	2.8	2.6	5.4

[a] Quantitated as rutin. [b] Quantitated as phloridzin.

Table IV. Influence of Extraction Procedure (Pressing vs Diffusion) on the Quercetin and Phloretin Glycoside Composition[a] (Milligrams per Liter) of Apple Juice

treatment	quercetin 3-O-[b] gal	glu	xyl	arab	rhamn	total quercetin glycosides	phloretin[c] xylglu	phloridzin	total phloretin glycosides
Red Delicious									
pressed (control)	0.0	0.0	0.0	0.0	0.0	0.0	4.3	11.8	16.1
diffusion									
55 °C	5.4	0.0	2.3	4.7	5.1	17.5	3.0	14.5	17.4
63 °C	10.2	0.0	4.8	8.5	7.8	31.2	6.4	32.3	38.7
67 °C	13.8	0.0	5.9	9.5	6.3	35.5	7.4	41.5	48.9
73 °C	14.4	0.0	6.3	10.6	6.8	38.2	8.7	50.4	59.1
McIntosh									
pressed (control)	0.0	0.0	0.0	0.0	0.0	0.0	6.0	8.7	14.7
diffusion									
55 °C	6.6	7.3	4.2	13.8	13.2	45.1	14.9	44.0	58.9
67 °C	8.0	9.1	4.9	16.0	13.8	51.8	18.6	56.0	74.6
Spartan									
pressed (control)	0.0	0.0	0.0	0.0	0.0	0.0	3.4	5.2	8.6
diffusion									
55 °C	5.2	2.0	2.7	5.2	2.3	17.4	3.9	17.7	21.6
63 °C	3.4	0.0	4.0	8.9	3.7	19.9	3.9	33.2	37.0
73 °C	8.2	1.6	3.7	10.3	6.1	29.9	8.8	36.2	45.0

[a] Data normalized to 11.5° Brix. [b] Quantitated as rutin. [c] Quantitated as phloridzin.

probably due to facilitated extraction of seeds and peels, enzyme inactivation, and increased solubility of quercetin and phloretin derivatives at the high temperatures of the diffusion process.

Influence of Processing and Storage on the Procyanidins. The procyanidin composition of Granny Smith apple juice and the changes that occur with processing and storage are shown in Table V. The differences in procyanidin composition between pressing and diffusion extraction at different temperatures for Red Delicious, McIntosh, and Spartan apple juices are shown in Table VI. Epicatechin levels are higher than catechin. The major procyanidin dimers are B2 (dimer of epicatechin) and B1 (dimer of epicatechin–catechin). Low levels of B3 and B4, dimers with alternative stereochemistry, are also present. Up to five unknown procyanidin peaks were detected in the different samples, and they are reported as total unknowns (by summation). These findings are in agreement with previous publications (Schmidt and Neukom, 1969; Lea, 1978, 1984) that report complex procyanidin composition, consisting of the dimeric procyanidin B2 up to the heptameric, where seven epicatechin units are joined together. Mixed procyanidins of epicatechin and catechin, and also the procyanidins of alternative stereochemistry, are also reported in these studies.

The data of Table V indicate that juice pressed from short-stored fruit contains higher levels of procyanidins than that from the long-stored fruit. The protection of procyanidins by HTST treatment is similar to that for cinnamics, and it is more pronounced in the juice extracted from the long-stored fruit. The highest procyanidin concentrations are found in the juice sampled at the HTST stage, and it appears that some losses of procyanidins occur during enzyme clarification, bottling, and concentration. The increased sensitivity of procyanidins to chemical oxidation at increased temperatures may account for the observed reductions. While there is evidence for some loss of procyanidins with fining, there is not a pronounced

Table V. Influence of Processing and Storage on the Procyanidin Composition[a] (Milligrams per Liter) of Granny Smith Apple Juice

treatment	B3	B1	B4	catechin	B2	trimer + tetramer	epicatechin	total unknowns[b]	total procyanidins[c]
short-stored fruit									
press	0.4	1.3	0.2	2.1	0.7	0.8	3.4	2.6	11.6
HTST	0.4	2.8	0.5	3.5	2.9	2.8	7.8	5.3	26.0
enzyme clarif	0.2	2.5	tr[d]	3.8	2.7	2.7	7.1	3.0	22.0
filtered, fined	tr	2.3	tr	3.3	2.7	2.5	6.9	5.1	23.0
bottled, fined	tr	2.2	tr	3.5	2.6	2.0	6.5	3.1	20.0
concentrated, fined	tr	1.9	0.7	2.9	2.4	2.8	6.1	4.1	21.0
concentrate, stored	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
filtered, not fined	0.7	2.6	0.9	3.4	2.8	2.9	7.9	3.3	24.5
bottled, not fined	tr	1.6	tr	2.5	1.9	1.6	4.9	2.3	15.0
concentrated, not fined	tr	2.3	0.2	3.0	2.7	2.7	6.0	4.1	21.0
concentrate, stored	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
long-stored fruit									
press	tr	tr	tr	0.2	0.2	tr	tr	0.1	0.7
HTST	0.2	1.6	0.1	1.9	2.1	2.1	4.4	2.9	15.4
enzyme clarif	tr	1.7	0.2	2.1	1.8	1.7	4.7	1.8	14.0
bottled, fined	tr	1.9	tr	1.7	1.7	1.4	3.5	1.3	11.6
concentrated, fined	tr	1.2	0.0	1.9	1.4	1.6	3.6	1.6	11.4
bottled, not fined	tr	1.8	tr	1.7	1.8	1.7	3.4	2.7	13.2
concentrated, not fined	tr	2.0	0.2	2.1	1.9	1.8	3.7	2.3	14.0

[a] Procyanidins B1, B2, B3, B4, trimer, tetramer, and total unknowns are quantitated as catechin. [b] Marked with × on Figure 3. [c] Includes catechin and epicatechin. [d] Less than 0.2 mg/L.

Table VI. Influence of Extraction Procedure (Pressing vs Diffusion Extraction) on the Procyanidin Composition[a] (Milligrams per Liter) of Apple Juice

treatment	B3	B1	B4	catechin	B2	trimer + tetramer	epicatechin	total unknowns[b]	total procyanidins[c]
Red Delicious									
pressed (control)	0.5	2.6	1.8	4.0	1.3	2.7	9.1	2.5	24.5
diffusion									
55 °C	0.0	1.2	1.1	2.0	1.1	1.5	4.2	1.5	12.6
63 °C	2.4	4.5	2.5	9.5	3.8	3.9	20.7	6.1	53.4
67 °C	1.3	5.1	2.8	10.1	2.1	7.7	24.9	10.5	64.5
73 °C	0.6	12.7	2.4	13.5	2.3	24.2	44.4	14.6	114.6
McIntosh									
pressed (control)	0.0	0.0	0.0	0.2	0.0	0.0	0.6	1.0	1.9
diffusion									
55 °C	0.0	1.5	1.1	4.0	0.0	2.6	6.6	7.1	22.9
67 °C	1.2	4.2	2.1	5.5	2.2	7.7	10.2	8.8	41.9
Spartan									
pressed (control)	0.0	0.0	0.0	1.2	0.0	0.0	1.6	0.0	2.8
diffusion									
55 °C	0.0	0.6	0.0	4.0	0.8	0.5	6.0	0.5	12.5
63 °C	0.0	0.5	0.0	1.0	0.0	0.5	7.5	0.0	9.6
73 °C	0.7	10.6	2.9	16.9	3.1	12.8	34.0	8.5	89.5

[a] Procyanidins B1, B2, B3, B4, trimer, tetramer, and total unknowns are quantitated as catechin; data normalized to 11.5° Brix. [b] Marked with × on Figure 3. [c] Includes catechin and epicatechin.

Table VII. Cinnamic and HMF Composition[a] (Milligrams per Liter) of Commercial Concentrates

sample[b]	HMF	chlorogenic	chlorogenic isomer[c]	caffeic	conjugated coumaric[c]	coumaryl-quinic[c]	coumaric	total cinnamics
A	5.1	44.9	3.9	2.3	2.8	17.2	1.0	72.0
B	7.2	48.5	3.7	2.2	2.6	18.0	0.8	75.8
C	7.9	26.7	2.5	1.5	1.5	9.7	0.7	42.6
D	4.9	0.9	0.0	0.0	0.0	0.0	0.1	1.1

[a] Data normalized to 11.5° Brix. [b] Samples C, D had been decolorized with activated carbon and then reconstituted. [c] Quantitated as chlorogenic acid.

Table VIII. Quercetin and Phloretin Glycoside Composition[a] (Milligrams per Liter) of Commercial Concentrates

	quercetin 3-*O*-[c]								
sample[b]	gal	glu	xyl	arab	rhamn	total quercetin glycosides	phloretin[d] xylglu	phloridzin	total phloretin glycosides
A	0.0	0.0	0.0	0.0	0.0	0.0	10.6	11.1	21.8
B	0.0	2.7	0.9	0.0	0.0	3.5	12.7	11.7	24.3
C	0.0	0.0	0.0	0.0	0.0	0.0	2.5	1.3	3.8
D	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a] Data normalized to 11.5° Brix. [b] Samples C, D had been decolorized with activated carbon and then reconstituted. [c] Quantitated as rutin. [d] Quantitated as phloridzin.

Table IX. Procyanidin Composition[a] (Milligrams per Liter) of Commercial Concentrates

sample[b]	B3	B1	B4	catechin	B2	trimer + tetramer	epicatechin	total unknowns[c]	total procyanidins[d]
A	0.0	0.5	0.4	0.9	0.3	0.5	0.3	0.3	3.3
B	0.0	0.5	0.3	0.5	0.0	0.0	0.7	0.0	2.1
C	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
D	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; data normalized to 11.5° Brix. [b] Samples C and D had been decolorized with activated carbon and then reconstituted. [c] Marked with × on Figure 3. [d] Includes catechin and epicatechin.

reduction in procyanidin concentrations as a result of that unit operation. Storage of concentrates for 9 months at 25 °C resulted in complete procyanidin degradation.

Procyanidin yield in diffusion-extracted juices is much higher than the procyanidin yield in conventional pressed juices (Table VI). The effect of diffusion extraction on procyanidin composition is more pronounced than the effect of diffusion extraction on the cinnamic composition. The reasons for the higher procyanidin yields with diffusion extraction are probably similar to the reasons for the increased yields in cinnamics previously discussed. The high sensitivity of procyanidins to oxidation, higher than that of cinnamics (Siegelman, 1955), probably accounts for the low procyanidin levels found in the juices from conventional pressing.

Analysis of Commercial Concentrates. The cinnamic and HMF composition of four commercial apple juice concentrates is shown in Table VII. Moderate levels of HMF were found in these samples relative to the stored concentrates of Granny Smith. Samples A and B contained significant amount of cinnamics, sample C had moderate levels, and sample D contained only a trace of cinnamics. Carbon (charcoal) treatment probably accounts for the low levels of phenolics in sample D. The quercetin composition of all samples (Table VIII) amounted to only traces, while samples A and B contained high levels of phloridzin. Procyanidin levels (Table IX) were very low, probably because of degradation during storage. The high levels of phloretin glycosides in samples A and B are not enough to lead one to suspect the possibility of diffusion extraction.

Total Phenolics by HPLC and by Colorimetric Assay. Table X shows the relation between the concentration of total phenolics as quantitated by HPLC and by the colorimetric procedure for all the juice samples analyzed. Some correlation ($r^2 = 0.874$) was obtained between the two methods when all the samples were considered as one group. The different groups of samples, however, showed considerable difference in the correlation of these two methods. HPLC and colorimetric assay are correlated highly ($r^2 = 0.973$) in the group of commercial samples, poorly ($r^2 = 0.823$) in the group of diffusion-extracted samples, and not at all ($r^2 = 0.003$) in the group of samples that consists of the different processing and storage stages of Granny Smith juice. HPLC is a specific method for quantitation of individual phenolic compounds, while the colorimetric procedure is a general assessment of the levels of phenolics. Degradation of phenolics occurring during processing and storage, as well as formation of browning intermediates, such as enediols and reductones (Van Buren et al., 1976), results in significant and variable interference with the colorimetric assay. HPLC quantitation in a sample that has undergone considerable phenolic degradation would give a lower concentration of phenolics, while colorimetric assay may show an even higher concentration of phenolics depending upon the levels and reactivity of the interfering compounds present. The commercial concentrates may be considered a similar group of samples in regard to the extent of phenolic degradation and presence of interfering compounds generated during storage. This similarity would allow a high correlation between the specific quantitation of HPLC and the less specific quantitation of the colorimetric assay. The different processing and storage

Table X. Total Phenolics by HPLC and by Colorimetric Assay (Milligrams per Liter)

variety/treatment	total phenolics[a] by HPLC	total phenolics by colorimetric assay as gallic acid	total phenolics by colorimetric assay as chlorogenic acid
Granny Smith			
short-stored fruit			
press	30.9	188	319
HTST	68.4	251	422
enzyme clarif	55.2	252	425
filtered, fined	58.4	216	365
bottled, fined	52.9	207	351
concentrated, fined	58.1	224	378
concentrate, stored	22.4	310	523
filtered, not fined	57.6	223	377
bottled, not fined	48.1	231	390
concentrated, not fined	55.4	229	385
concentrate, stored	17.2	316	525
long-stored fruit			
press	12.5	142	244
HTST	54.5	228	384
enzyme clarif	43.6	217	368
bottled, fined	39.3	178	303
concentrated, fined	47.2	211	356
bottled, not fined	47.4	219	370
concentrated, not fined	44.1	232	391
Red Delicious			
pressed (control)	132.3	374	622
diffusion			
55 °C	101.5	401	672
63 °C	218.0	579	963
67 °C	253.7	624	1037
73 °C	332.8	780	1292
McIntosh			
pressed (control)	66.8	160	274
diffusion			
55 °C	268.1	483	801
67 °C	330.2	580	961
Spartan			
pressed (control)	69.7	176	298
diffusion			
55 °C	116.5	272	457
63 °C	196.3	502	833
73 °C	310.8	567	939
commercial concentrates			
A	97.1	204	341
B	105.8	224	374
C	46.7	113	193
D	1.1	49	88

[a] Includes cinnamics, quercetin and phloretin glucosides, and procyanidins.

stages for the Granny Smith apple juice lack uniformity, and as a result there is no correlation between the two methods.

CONCLUSION

There are considerable quantitative differences in the phenolic composition of apple juices from different varieties. In addition to variety, processing techniques affect the level of phenolics present in the finished juices. HTST treatment immediately after pressing effectively protected the phenolics extracted in the juice from PPO. Enzymatic clarification caused hydrolysis of conjugated cinnamics. This was less apparent in fined samples or in bottled juices. Long-term storage of fruit resulted in lower concentration of procyanidins. Fining had little effect on the procyanidin profile. Some loss of procyanidins was observed with clarification, bottling, and concentration. Storage of concentrates resulted in formation of HMF, loss of approximately 36% of cinnamics, loss of 50–60% of quercetin and phloretin derivatives, and total loss of procyanidins. Diffusion-extracted juice contained increased levels of phenolics relative to juice processed with conventional pressing. It appears that better extraction and less oxidation of phenolics occur as the temperature of extraction increases. The effect of diffusion extraction on the procyanidin, quercetin glycoside, and phloretin glycoside composition was more pronounced than the effect of diffusion on the cinnamic composition. The phenolic profile, especially the levels of phloretin and quercetin derivatives, may provide an indicator for diffusion extraction. The concentration of phenolics obtained by the Folin–Ciocalteu procedure should be interpreted with caution as nonphenolic material can interfere with the assay.

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Registry No. HMF, 25376-49-2; quercetin 3-*O*-galactoside, 482-36-0; quercetin 3-*O*-glucoside, 482-35-9; quercetin 3-*O*-xyloside, 27214-55-7; quercetin 3-*O*-arabinoside, 572-30-5; quercetin 3-*O*-rhamnoside, 522-12-3; phloretin xyloglucoside, 58769-37-2; procyanidin B_1, 20315-25-7; procyanidin B_2, 29106-49-8; procyanidin B_3, 23567-23-9; procyanidin B_4, 29106-51-2; catechin, 154-23-4; epicatechin, 490-46-0; chlorogenic acid, 327-97-9; caffeic acid, 331-39-5; coumarylquinic acid, 34214-76-1; coumaric acid, 7400-08-0; phloridzin, 60-81-1.