Influence of Variety, Maturity, Processing, and Storage on the Phenolic Composition of Pear Juice†

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The influence of SO_2, initial high-temperature short-time (HTST) heat treatment, enzymatic clarification, fining, bottling, concentration, and storage on the phenolic composition of pear juice pressed from "hard" and "soft" Comice, d'Anjou, and Bartlett fruit was studied by means of HPLC with diode array detection. Variety and maturity introduced major quantitative differences in the phenolic profile. Considerable loss of cinnamics and total disappearance of procyanidins occurred in the juices processed without SO_2. Initial HTST treatment protected cinnamics and procyanidins from degradation during processing. Hydrolysis of cinnamics occurred during enzymatic clarification of juices; this hydrolytic activity ceased with bottling. There was no apparent reduction of phenolics with fining. Procyanidins were sensitive to the heat applied during bottling and concentration. Arbutin and flavonol glycosides were less affected by SO_2 and processing. Storage of concentrates for 9 months at 25 °C resulted in formation of low levels of (hydroxymethyl)furfural, extensive degradation of cinnamics and flavonol glycosides, and total loss of procyanidins.

Browning of pear juice and juice concentrate has been a major problem in the processing and marketing of pear juice products (Montgomery and Petropakis, 1980). While the color deterioration occurring during storage is primarily due to the Maillard reaction (Cornwell and Wrolstad, 1981), the development of brown color during processing is caused by oxidation of the phenolic compounds by polyphenoloxidase (PPO) present in the pear fruit (Rivas and Whitaker, 1973; Halim and Montgomery, 1978). The major phenolic constituents, other than arbutin (Williams, 1957), that have been reported in pears are cinnamic acids, such as chlorogenic and coumarylquinic acid (Siegelman, 1955; Sioud and Luh, 1966; Ranadive and Haard, 1971), glycosides of the flavonols quercetin and isorhamnetin such as rutin and isorhamnetin 3-glucoside (Nortjé and Koeppen, 1965; Duggan, 1969a), and catechins such as epicatechin and procyanidins (Sioud and Luh, 1966). Most of the reports, however, are qualitative rather than quantitative, and very few studies characterize the changes of individual phenolic compounds during processing.

In this study HPLC separation with diode array detection was used to examine the influence of processing and storage on the phenolic composition of pear juice. As it is common commercial practice to process pear juice from both hard green (unripe) and soft (ripened) fruit, the influence of maturity was included. The effect of SO_2 addition was studied because of its effectiveness as an inhibitor of PPO and possible improvement in product quality. Comparison of total phenolics determinations by HPLC and colorimetric assay was also done.

MATERIALS AND METHODS

Standards. Phenolic standards (chlorogenic, caffeic, and *p*-coumaric acids, protocatechuic acid, arbutin, rutin, catechin, epicatechin) and (hydroxymethyl)furfural (HMF) were obtained from Sigma Chemical Co. *p*-Coumarylquinic acid was previously isolated from apple juice (Spanos et al., 1990), procyanidin standards (B1, B2, B3, B4, trimer, tetramer) were donated by Dr. A. G. H. Lea of Cadbury Schweppes Ltd., Lord Zuckerman Research Center, University of Whiteknights, U.K. All solvents used were HPLC grade.

Preparation of Pear Juices. Comice, d'Anjou, and Bartlett pears were obtained in fall 1986, from the Mid-Columbia Experiment Station, Hood River, OR. Hard green (unripe) and soft (ripened) fruit was processed into juice in the pilot plant of the Department of Food Science, Oregon State University. Comice and d'Anjou juices extracted from hard fruit were processed with and without the addition of SO_2. All processing trials were replicated. Figure 1 shows a schematic outline of the steps utilized in processing. Juice was sampled at the intermediate processing stages as indicated on Figure 1 and stored at −30 °C until analysis. Concentrates that had been kept frozen or stored for 9 months at 25 °C were diluted to the original degrees Brix before analysis. Additional information concerning the processing of pear juice is reported elsewhere (Wrolstad et al., 1989).

Determination of Pear 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) were used. Coumarylquinic acid and an isomer of chlorogenic acid were quantitated as chlorogenic acid, flavonol glycosides were quantitated as rutin, and procyanidin quantitation was based on the standard curve of catechin. All HPLC analyses were replicated, the mean values being reported; reproducibility was ca. ±5%.

Dilutions 1/0, 1/2, 1/4, and 1/6 of a water solution containing 80 mg/L chlorogenic acid, 25 mg/L caffeic acid, 10 mg/L coumaric acid, 12 mg/L rutin, 25 mg/L arbutin, and 25 mg/L HMF were used to prepare the standard curves of these compounds. Similar dilutions of a water solution of 80 mg/L of catechin and 80 mg/L epicatechin were used for the quantitation of catechins and procyanidins.

Isolation and Hydrolysis of Chromatographic Peaks. The phenolic constituents of pear juice were concentrated (ca. 5-fold) by means of absorption on a C_{18} Sep-Pak, elution of sugars and nonvolatile acids with water, and subsequent elution of the phenolics with methanol. The methanol was evaporated, and the phenolic compounds were redissolved in water. Individual HPLC peaks were isolated from multiple injections of the isolate and subjected to alkaline hydrolysis as previously described (Spanos et al., 1990).

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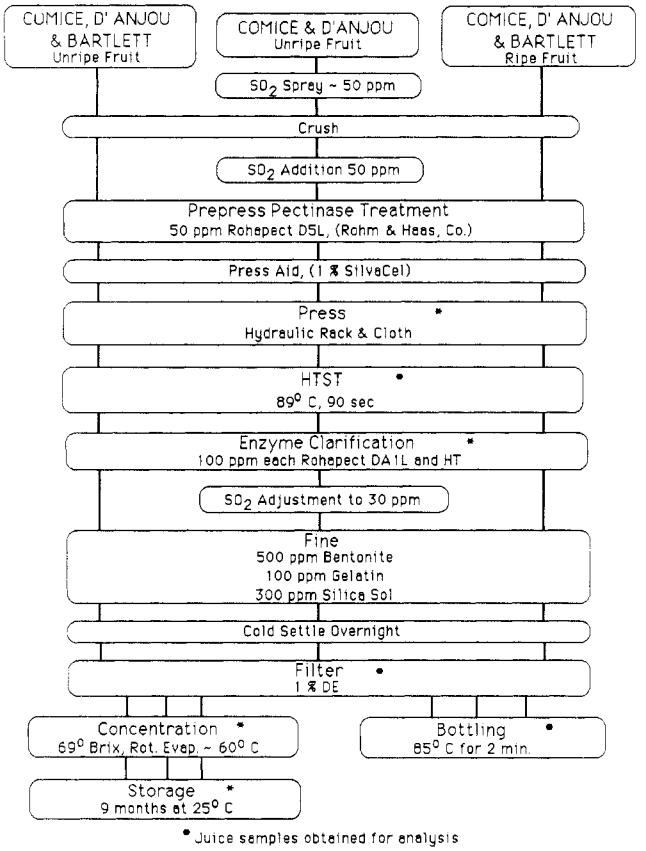

Figure 1. Outline of steps utilized in processing of pear juice.

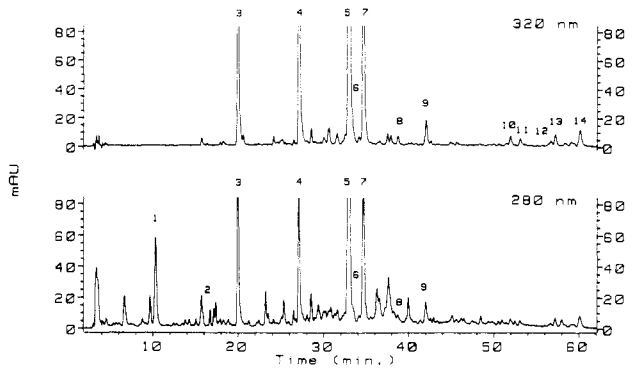

Figure 2. HPLC chromatogram of pear juice cinnamics, flavonols, and arbutin. Peaks: 1, arbutin; 2, HMF; 3, oxidized cinnamic; 4, oxidized cinnamic; 5, chlorogenic; 6, chlorogenic isomer; 7, caffeic; 8, coumarylquinic; 9, coumaric; 10, rutin; 11, quercetin galactoside; 12–14, isorhamnetin glycosides.

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 Phenolic Compounds. A typical HPLC chromatogram of cinnamics, flavonol glycosides, arbutin, and HMF from pear juice is shown in Figure 2. Chlorogenic, caffeic, coumaric, and coumarylquinic acids (peaks 5, 7, 8, and 9, respectively) were identified by matching both retention data and spectral characteristics of the corresponding peaks with those of standards. Similarly, the presence of arbutin (peak 1), HMF (peak 2), and rutin (peak 10) was verified by both retention times and spectral data. Peak 6 was tentatively identified as an isomer of chlorogenic acid by comparing its spectral and retention data with those of an isomer of

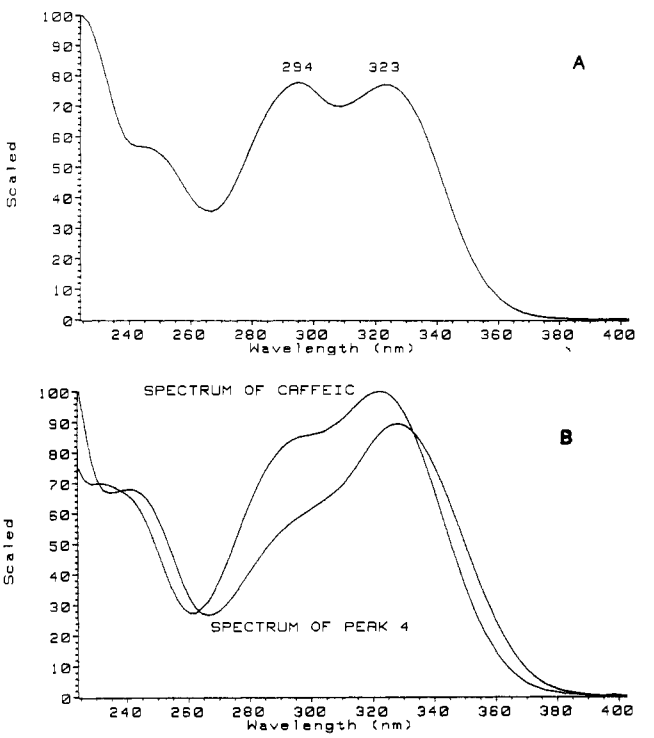

Figure 3. UV spectra: (A) peak 3 (corresponding to Figure 2); (B) caffeic acid and peak 4 (corresponding to Figure 2).

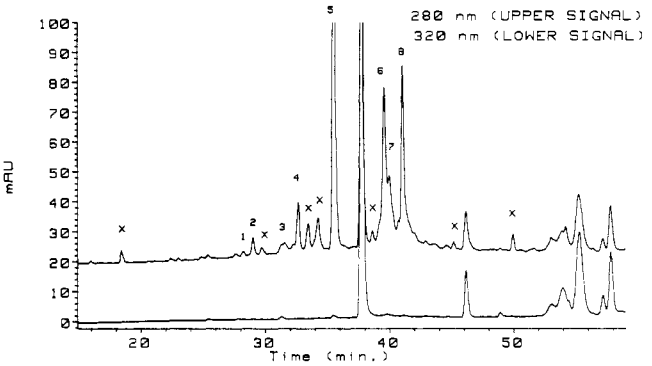

Figure 4. HPLC separation of pear juice procyanidins. Peaks: 1, procyanidin B3; 2, procyanidin B1; 3, procyanidin B4; 4, catechin; 5, procyanidin B2; 6, trimer; 7, tetramer; 8, epicatechin; x, unknown procyanidins.

chlorogenic acid found in apples (Spanos et al., 1990). Peak 11 was tentatively identified as quercetin galactoside. It exhibited a quercetin spectrum and its retention time corresponded to quercetin galactoside, which is present in apple juice (Spanos et al., 1990). Peaks 12–14 were assigned as glycosides of isorhamnetin. They had typical quercetin spectra, but their longer retention (longer than any quercetin derivative) suggested a more hydrophobic structure. Isorhamnetin has the same chromophore as quercetin, but it is more hydrophobic because of the methyl group at the 3′-position. The galactoside, glucoside, rutinoside, and glucorutinoside derivatives of isorhamnetin have been reported in pear fruits (Nortjé and Koeppen, 1965; Duggan, 1969a); the presence of isorhamnetin aglycon in hydrolyzed pear extracts has also been reported (Duggan, 1969b).

Peaks 3 and 4 were not completely characterized. They were major peaks in the chromatograms of SO_2-processed pear juices from Comice and d'Anjou fruit, and they were fairly stable during the storage of the concentrates. They were not detectable, however, in any pear juice processed without SO_2. They were extracted from

Table I. Influence of Processing and Storage on the Cinnamic and HMF Composition (mg/L) of Comice Pear Juice

treatment	HMF	oxidized cinnamics[a] A	oxidized cinnamics[a] B	chlorogenic	chlorogenic isomer[b]	caffeic	coumarylquinic[b]	coumaric	total cinnamics[c]
$-SO_2$ unripe									
press	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.4	0.6
HTST	0.0	0.0	0.0	9.2	0.0	0.3	0.7	0.4	10.6
enzyme clarif	0.0	0.0	0.0	4.1	0.0	2.3	0.0	0.9	7.3
fined, filtered	0.0	0.0	0.0	2.6	0.0	2.4	0.0	0.8	5.7
bottled	0.0	0.0	0.0	4.2	0.0	1.5	0.0	0.6	6.4
concentrated	0.0	0.0	0.0	2.5	0.0	2.7	0.0	0.8	6.0
concentrate, stored	4.2	0.0	0.0	1.8	0.0	1.2	0.0	0.5	3.5
$+SO_2$ unripe									
press	0.0	21.9	27.2	34.8	0.0	5.2	0.8	1.2	91.1
HTST	0.0	20.7	23.1	89.9	0.6	1.8	1.9	0.5	138.4
enzyme clarif	0.0	21.0	24.7	62.5	0.6	13.9	1.0	1.3	125.0
fined, filtered	0.0	17.8	23.1	61.7	0.3	12.5	0.6	1.0	117.0
bottled	0.0	18.7	23.0	69.7	1.0	7.6	1.0	1.0	121.9
concentrated	0.0	19.0	22.5	53.8	0.6	17.1	0.7	1.3	115.0
concentrate, stored	3.8	14.5	20.0	14.5	0.3	18.4	0.9	1.4	69.9
$-SO_2$ ripe									
press	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
HTST	0.0	0.0	0.0	65.1	0.5	0.8	1.8	0.4	68.6
enzyme clarif	0.0	0.0	0.0	27.7	0.2	18.8	0.0	1.9	48.6
fined	0.0	0.0	0.0	34.9	0.2	12.3	0.0	1.7	49.2
bottled	tr[d]	0.0	0.0	41.8	0.9	8.3	0.2	1.7	52.8
concentrated	0.0	0.0	0.0	35.9	0.8	13.1	0.3	1.7	51.7
concentrate, stored	3.1	0.0	0.0	10.8	0.0	12.4	0.0	0.0	23.1

[a] Compounds A and B correspond to peaks 4 and 5, respectively, of Figure 4, quantitated as chlorogenic acid. [b] Quantitated as chlorogenic acid. [c] Includes oxidized cinnamics. [d] Less than 0.2 mg/L.

Table II. Influence of Processing and Storage on the Cinnamic and HMF Composition (mg/L) of d'Anjou Pear Juice

treatment	HMF	oxidized cinnamics[a] A	oxidized cinnamics[a] B	chlorogenic	chlorogenic isomer[b]	caffeic	coumarylquinic[b]	coumaric	total cinnamics[c]
$-SO_2$ unripe									
press	0.0	0.0	0.0	0.7	0.0	0.5	0.0	0.6	1.8
HTST	0.0	0.0	0.0	12.2	0.0	0.6	0.9	0.6	14.4
enzyme clarif	0.0	0.0	0.0	4.4	1.0	3.4	0.0	1.9	10.7
fined, filtered	0.0	0.0	0.0	5.1	0.6	2.2	0.0	1.0	8.9
bottled	0.0	0.0	0.0	6.7	0.8	1.6	0.0	1.3	10.3
concentrated	tr[d]	0.0	0.0	4.7	0.9	2.9	0.3	1.5	10.3
concentrate, stored	5.1	0.0	0.0	1.1	0.6	2.3	0.0	1.5	5.5
$+SO_2$ unripe									
press	0.0	13.2	12.9	126.5	1.5	14.2	2.7	1.7	172.6
HTST	0.0	12.5	12.2	156.8	1.7	2.1	3.7	0.8	189.8
enzyme clarif	0.0	11.7	12.8	116.5	1.6	18.4	2.0	1.9	164.9
fined, filtered	0.0	10.6	12.3	117.9	1.5	17.0	1.9	1.8	163.1
bottled	tr	11.1	12.8	131.3	1.8	9.1	2.1	1.7	169.9
concentrated	0.0	11.4	12.5	116.3	1.3	17.9	1.1	2.1	162.7
concentrate, stored	8.6	8.5	13.0	41.6	1.4	25.4	0.0	2.2	92.1
$-SO_2$ ripe									
press	0.0	0.0	0.0	1.0	0.7	0.7	0.0	0.4	2.7
HTST	0.0	0.0	0.0	84.1	2.0	1.0	3.0	0.7	90.8
enzyme clarif	0.0	0.0	0.0	42.6	1.6	22.0	0.6	2.5	69.4
fined, filtered	0.0	0.0	0.0	59.1	1.5	11.3	0.8	2.0	74.7
bottled	0.0	0.0	0.0	60.7	2.1	8.8	1.3	2.0	75.0
concentrated	tr	0.0	0.0	56.8	1.9	12.8	1.1	2.5	75.1
concentrate, stored	9.4	0.0	0.0	24.4	0.8	13.0	0.3	1.5	40.0

[a] Compounds A and B correspond to peaks 4 and 5, respectively, of Figure 4, quantitated as chlorogenic acid. [b] Quantitated as chlorogenic acid. [c] Includes oxidized cinnamics. [d] Less than 0.2 mg/L.

juice to a small degree in ethyl acetate, indicating that they are polar compounds. Their UV spectra are shown in parts A and B of Figure 3. Peaks with similar spectra but shorter retention times have been previously reported in Thompson seedless grape juice (Spanos and Wrolstad, 1990). A UV spectrum similar to that of Figure 3A was obtained through summation of a normalized spectrum of a cinnamic acid (caffeic acid) and a hydroxybenzoic acid (protocatechuic acid). The UV spectrum of peak 4 exhibits some similarities with the spectrum of caffeic acid (Figure 3B). If these compounds were esters of caffeic acid, one would expect the ester linkage to be cleaved with alkaline hydrolysis and release of caffeic acid. Attempts to detect a cinnamic or a hydroxybenzoic acid as a product of alkaline or acid hydrolysis of the isolated peaks, however, were not successful. Degradation of these compounds with alkaline hydrolysis conditions was less than for chlorogenic acid under the same conditions. Because of the stability of these compounds during storage and their resistance to hydrolysis, it is unlikely that they were present in the juices processed without SO_2. Their precursors could have been rapidly degraded in the absence of SO_2. It seems likely that they are intermediate oxidation products of chlorogenic or caffeic acid, with SO_2 allowing for their formation. Their UV spectra are modified spectra of chlorogenic or caffeic acid. These compounds do not readily undergo further oxidation, and this could explain the clear white color of the

Table III. Influence of Processing and Storage on the Cinnamic and HMF Composition (mg/L) of Bartlett Pear Juice

treatment	HMF	oxidized cinnamics[a] A	oxidized cinnamics[a] B	chlorogenic	chlorogenic isomer[b]	caffeic	coumarylquinic[b]	coumaric	total cinnamics[c]
$-SO_2$ unripe									
press	0.0	0.0	0.0	0.0	0.0	0.0	0.0	tr[d]	0.0
HTST	0.0	0.0	0.0	3.3	2.1	0.4	0.0	0.5	6.2
enzyme clarif	0.0	0.0	0.0	0.0	2.1	1.7	0.0	0.8	4.5
fined, filtered	0.0	0.0	0.0	1.4	0.0	1.1	0.0	tr	2.3
bottled	0.0	0.0	0.0	2.9	0.0	1.2	0.0	0.3	4.4
concentrated	0.0	0.0	0.0	0.7	0.6	1.1	0.0	0.4	2.9
concentrate, stored	6.9	0.0	0.0	tr	0.0	0.3	0.0	0.4	0.8
$-SO_2$ ripe									
press	0.0	0.0	0.0	0.4	0.0	1.1	0.0	0.5	1.9
HTST	0.0	0.0	0.0	4.6	0.0	tr	0.0	0.3	4.9
enzyme clarif	0.0	0.0	0.0	0.6	0.0	3.1	0.0	2.7	6.4
fined, filtered	0.0	0.0	0.0	1.7	2.1	2.8	0.0	2.2	8.8
bottled	0.0	0.0	0.0	0.6	0.0	1.2	0.0	0.7	2.4
concentrated	0.0	0.0	0.0	0.6	1.0	2.7	0.0	2.1	6.4
concentrate, stored	5.1	0.0	0.0	0.0	0.0	1.0	0.0	tr	1.0

[a] Compounds A and B correspond to peaks 4 and 5, respectively, of Figure 4, quantitated as chlorogenic acid. [b] Quantitated as chlorogenic acid. [c] Includes oxidized cinnamics. [d] Less than 0.2 mg/L.

Table IV. Influence of Processing and Storage on the Arbutin and Flavonol Composition (mg/L) of Comice Pear Juice

treatment	arbutin[a]	rutin	quercetin galactoside[b]	isorhamnetin glycosides[b] A	isorhamnetin glycosides[b] B	isorhamnetin glycosides[b] C	total flavonols
$-SO_2$ unripe							
press	13.5	tr[c]	1.2	0.0	1.3	2.6	5.1
HTST	13.0	1.1	1.4	tr	1.3	2.6	6.4
enzyme clarif	14.3	1.2	1.4	tr	1.4	2.5	6.5
fined, filtered	13.7	1.0	1.3	tr	1.2	2.7	6.2
bottled	12.9	1.0	1.1	tr	1.1	2.6	5.8
concentrated	13.5	1.1	1.3	tr	1.0	2.9	6.3
concentrate, stored	14.2	0.0	tr	0.0	tr	1.1	1.0
$+SO_2$ unripe							
press	17.0	1.8	1.8	1.1	2.1	3.1	9.9
HTST	16.1	2.5	3.6	1.1	2.3	3.0	12.4
enzyme clarif	16.4	2.4	3.3	1.3	1.9	3.2	12.1
fined, filtered	16.0	1.9	4.4	1.0	2.1	3.2	12.6
bottled	16.8	3.0	1.4	1.1	2.0	2.9	10.4
concentrated	15.7	2.7	2.3	1.2	2.1	2.8	11.1
concentrate, stored	18.8	0.0	1.4	0.0	1.0	2.1	4.5
$-SO_2$ ripe							
press	16.6	0.0	0.0	tr	1.6	2.3	3.9
HTST	17.0	1.8	1.8	1.1	1.7	2.1	8.5
enzyme clarif	16.7	2.7	2.6	1.0	1.6	2.0	9.9
fined, filtered	16.5	2.0	1.8	1.2	1.9	1.5	8.5
bottled	16.1	2.2	2.6	1.1	1.5	1.9	9.4
concentrated	15.8	1.9	1.6	1.2	1.3	2.1	8.1
concentrate, stored	16.8	2.4	0.0	0.0	tr	1.1	3.4

[a] Interference present in the quantitation of arbutin in the stored concentrates. [b] Quantitated as rutin. [c] Less than 0.9 mg/L.

SO_2-processed juice relative to the brown color of the juice processed without SO_2. A similar mechanism preventing the formation of brown compounds is believed to take place in grapes where caftaric acid is oxidized to the relatively stable and colorless glutathionylcaftaric acid (Singleton et al., 1985; Cheynier et al., 1986; Singleton, 1987; Cheynier and Van Hulst, 1988). Stable colorless compounds from phenolic oxidation in the presence of sulfites and other sulfhydryl compounds have been reported in a number of studies (Walker, 1964; Walker and Reddish, 1964; Montgomery, 1983; Singleton, 1987).

An HPLC chromatogram of procyanidins isolated with Sephadex LH-20 from pear juice is shown in Figure 4. Identification of catechin, epicatechin, and procyanidins B1, B2, B3, B4, trimer, and tetramer was based on retention times of standards and on the typical catechin spectrum. A number (up to eight) of minor peaks also exhibited typical catechin spectra, and they were considered to be unknown procyanidins.

Effect of Variety, Maturity, Processing, and Storage on Cinnamics. The influence of processing and storage on the cinnamic composition of Comice, d'Anjou, and Bartlett pear juice is shown in Tables I–III. SO_2, fruit maturity, and variety have apparent influence on the cinnamic composition of pear juice. The data (Tables I and II) on Comice and d'Anjou juices from unripe fruit, which was processed both with and without SO_2, illustrate the effect of sulfites on the cinnamic composition. Significant cinnamic oxidation occurred in juices from both varieties that were not protected from oxidation by the reducing agent. Direct inhibition of PPO and reduction of the quinones generated upon PPO activity on cinnamics are the mechanisms that retard phenolic degradation in the presence of SO_2 (Mayer and Harel, 1979). The presence of SO_2 also resulted in formation of compounds believed to be stable intermediate cinnamic oxidation products, as previously discussed. Increased levels of cinnamics were found in the pear juices pressed from Comice and d'Anjou soft (ripe) fruit relative to those from hard (unripe) fruit. Comparison between juice from ripe and unripe fruit is made at the zero SO_2 level as ripe fruit was processed only without SO_2. Increase in chlorogenic acid

Table V. Influence of Processing and Storage on the Arbutin and Flavonol Composition (mg/L) of d'Anjou Pear Juice

treatment	arbutin[a]	rutin	quercetin galactoside[b]	isorhamnetin glycosides[b] A	B	C	total flavonols
$-SO_2$ unripe							
press	6.5	tr[c]	tr	0.0	tr	1.3	1.3
HTST	6.3	1.3	tr	0.0	tr	1.5	2.8
enzyme clarif	6.8	1.1	tr	0.0	tr	1.1	2.2
fined, filtered	6.1	tr	tr	0.0	tr	tr	0.0
bottled	6.5	tr	tr	0.0	tr	1.2	1.2
concentrated	7.0	tr	tr	0.0	tr	1.2	1.2
concentrated, stored	10.3	0.0	0.0	0.0	tr	tr	0.0
$+SO_2$ unripe							
press	7.9	3.1	1.9	0.0	tr	1.7	6.6
HTST	8.1	3.6	1.6	0.0	tr	2.5	7.7
enzyme clarif	7.7	2.3	2.3	0.0	tr	2.8	7.4
fined, filtered	8.0	3.6	2.0	0.0	tr	2.1	7.7
bottled	6.7	3.6	1.8	0.0	tr	2.2	7.6
concentrated	7.2	3.3	1.2	0.0	tr	1.8	6.3
concentrated, stored	10.8	2.1	tr	0.0	0.0	0.0	2.1
$-SO_2$ ripe							
press	7.6	0.0	0.0	0.0	tr	tr	0.0
HTST	8.9	5.1	2.0	0.0	tr	tr	7.1
enzyme clarif	9.0	5.1	2.2	0.0	tr	tr	7.3
fined, filtered	8.5	4.6	2.0	0.0	tr	tr	6.6
bottled	8.6	5.0	1.4	0.0	tr	tr	6.4
concentrated	8.1	4.9	2.0	0.0	tr	tr	6.9
concentrated, stored	16.4	2.3	tr	0.0	0.0	0.0	2.2

[a] Interference present in the quantitation of arbutin in the stored concentrates. [b] Quantitated as rutin. [c] Less than 0.9 mg/L.

Table VI. Influence of Processing and Storage on the Arbutin and Flavonol Composition (mg/L) of Bartlett Pear Juice

treatment	arbutin[a]	rutin	quercetin galactoside[b]	isorhamnetin glycosides[b] A	B	C	total flavonols
$-SO_2$ unripe							
press	10.8	tr[c]	tr	tr	tr	1.5	1.5
HTST	9.9	1.1	tr	0.0	1.2	1.6	3.9
enzyme clarif	10.1	tr	tr	0.0	1.1	1.3	2.4
fined, filtered	9.5	tr	tr	0.0	tr	1.6	1.6
bottled	9.4	tr	tr	0.0	tr	tr	0.0
concentrated	9.7	tr	tr	0.0	tr	tr	0.0
concentrate, stored	12.0	0.0	0.0	0.0	0.0	0.0	0.0
$-SO_2$ ripe							
press	7.7	tr	0.0	0.0	tr	tr	0.0
HTST	6.7	tr	0.0	0.0	tr	1.4	1.4
enzyme clarif	7.2	tr	0.0	0.0	tr	1.6	1.6
fined, filtered	7.4	tr	0.0	0.0	tr	1.2	1.2
bottled	7.9	tr	0.0	0.0	tr	1.8	1.8
concentrated	7.2	tr	0.0	0.0	tr	1.4	1.4
concentrate, stored	8.1	0.0	0.0	0.0	0.0	0.0	0.0

[a] Interference present in the quantitation of arbutin in the stored concentrates. [b] Quantitated as rutin. [c] Less than 0.8 mg/L.

from 50 μg/g (fresh weight) to 170 μg/g with storage of d'Anjou fruit at 1 °C for 170 days has been reported (Meadows, 1983). Varietal comparisons show that d'Anjou juice contains higher levels of cinnamics than Comice juice, while very low levels were present in Bartlett juice.

Processing also has an important effect on cinnamics. The minimum cinnamic levels were found in juice sampled at the press stage, while the maximum cinnamic levels occurred in the juices sampled at the HTST stage. It is known that PPO activity causes drastic phenolic oxidation, which 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; Spanos et al., 1990). Juice sampled at the press stage did not receive the HTST treatment that would inactivate the enzyme; hence, the degradation of cinnamics continued. While the press stage data do not accurately represent the actual concentration at that stage of processing, they reveal the effectiveness of HTST treatment in stopping PPO activity.

Hydrolysis of chlorogenic acid with enzymatic clarification was evident in juice processed from either ripe or unripe fruit regardless of the presence of SO_2. The compositional data at the clarification stage show lower levels of chlorogenic acid and higher levels of caffeic acid relative to those at HTST. The reduction in chlorogenic acid is in agreement with the increase in caffeic acid, as the standard curves of these compounds indicate that for every 1 mg of chlorogenic hydrolyzed approximately 0.5 mg of caffeic should be generated. Less loss of chlorogenic acid in bottled samples suggests that hydrolysis of cinnamics continues after clarification, but it can be deactivated with the pasteurization of bottling. Fining of juice pressed from ripe fruit also seemed to remove hydrolytic activity on chlorogenic acid.

Storage of concentrates for 9 months at 25 °C resulted in approximately 50–60% degradation of cinnamics in all the juices. (Hydroxymethyl)furfural (HMF) (up to 9.4 mg/L) was also formed during storage. The levels of HMF, however, found in the stored concentrates of pear juice are much lower than those in concentrates of grape or apple juice stored under the same conditions (Spanos and Wrolstad, 1990; Spanos et al., 1990).

Effect of Variety, Maturity, Processing, and Stor-

Table VII. Influence of Processing and Storage on the Procyanidin[a] Composition (mg/L) of Pear Juice

treatment	B3	B1	B4	catechin	B2	trimer + tetramer	epicatechin	total unknowns[b]	total procyanidins[c]
					Comice				
$-SO_2$ unripe	no procyanidins or catechins detected in any processing stage								
$+SO_2$ unripe									
press	tr[d]	0.6	0.4	1.5	6.8	8.7	6.2	3.1	27.2
HTST	tr	1.5	1.7	3.5	28.0	26.7	25.6	11.1	98.1
enzyme clarif	tr	1.3	1.7	3.3	26.4	25.2	22.0	10.6	90.6
fined, filtered	tr	1.5	1.6	3.3	27.5	24.3	18.4	9.7	86.3
bottled	tr	1.0	1.3	3.1	22.0	20.6	19.0	7.2	74.2
concentrated	tr	1.2	1.3	3.5	23.3	20.5	14.8	7.6	72.3
concentrate, stored	0.0	0.0	0.0	0.0	0.0	0.0	0.0	tr	0.0
$-SO_2$ ripe	no procyanidins or catechins detected in any processing stage								
					d'Anjou				
$-SO_2$ unripe	no procyanidins or catechins detected in any processing stage								
$+SO_2$ unripe									
press	tr	0.7	0.6	2.0	3.9	3.1	6.5	0.2	17.0
HTST	tr	1.0	1.6	2.9	5.2	4.4	8.4	1.2	24.9
enzyme clarif	tr	0.7	1.3	2.4	4.5	3.9	7.1	1.0	21.2
fined, filtered	tr	0.9	1.6	2.6	5.2	4.5	8.3	1.4	24.6
bottled	tr	tr	0.8	1.5	2.4	1.1	4.3	1.2	11.3
concentrated	tr	0.4	1.1	1.6	2.7	3.6	6.2	0.8	16.4
concentrate, stored	0.0	0.0	0.0	0.0	0.0	0.0	0.0	tr	0.0
$-SO_2$ ripe	no procyanidins or catechins detected in any processing stage								
					Bartlett				
$-SO_2$ unripe	no procyanidins or catechins detected in any processing stage								
$-SO_2$ ripe	no procyanidins or catechins detected in any processing stage								

[a] Procyanidins B1, B2, B3, B4, trimer, tetramer, and total unknowns are quantitated as catechin. [b] Marked with × on Figure 5. [c] Includes catechin and epicatechin. [d] Less than 0.2 mg/L.

Table VIII. Total Phenolics by HPLC[a] (mg/L) and by Colorimetric Assay (ppm Gallic Acid)

	Comice		d'Anjou		Bartlett	
treatment	HPLC	colorimetric assay	HPLC	colorimetric assay	HPLC	colorimetric assay
$-SO_2$ unripe						
press	19.2	205	9.6	138	12.6	177
HTST	30.0	239	23.5	159	20.0	198
enzyme clarif	28.1	223	19.7	152	16.9	206
fined, filtered	25.7	190	14.9	139	13.9	200
bottled	25.1	187	18.0	134	13.8	187
concentrated	25.8	197	18.4	147	12.5	208
concentrate, stored	18.7	247	15.7	219	12.7	386
$+SO_2$ unripe						
press	145.1	406	204.2	366		
HTST	265.1	542	230.4	378		
enzyme clarif	244.1	519	201.1	361		
fined, filtered	232.0	513	203.3	352		
bottled	223.4	464	195.6	332		
concentrated	214.1	475	192.6	351		
concentrate, stored	93.3	484	104.9	400		
$-SO_2$ ripe						
press	20.4	238	10.3	187	9.6	225
HTST	94.1	318	106.8	270	13.0	239
enzyme clarif	75.2	350	85.7	264	15.2	254
fined, filtered	74.2	271	89.7	228	17.4	210
bottled	78.3	305	90.0	239	12.1	227
concentrated	75.7	318	90.0	250	15.0	226
concentrate, stored	43.3	356	58.7	352	9.0	495

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

age on Arbutin and Flavonol Glycosides. Tables IV–VI show the arbutin and flavonol glycoside composition of Comice, d'Anjou, and Bartlett pears. The levels of arbutin in Comice juice are higher than those of d'Anjou and Bartlett juice. d'Anjou and Bartlett have similar arbutin contents. Slight increases in arbutin levels were measured in Comice and d'Anjou juice pressed from ripe fruit. Bartlett juice from ripe fruit, however, contained a slightly lower arbutin concentration than juice from unripe juice. The influence of SO_2 on arbutin is minor relative to the influence of SO_2 on cinnamics. Processing resulted in no apparent changes in the levels of arbutin. Storage of concentrates for 9 months at 25 °C resulted in formation of a compound interfering with the quantitation of arbutin as evidenced by the UV spectra of the peak that had the retention time of arbutin. Consequently, the data on arbutin levels do not represent the effect of storage on arbutin.

The levels of quercetin and isorhamnetin glycosides in most of the juices were close to the quantitation limit (0.8 mg/L). Apparent changes at such a low levels cannot be described.

Although the phenolic profile of pear juice has many qualitative similarities with the phenolic profile of apple juice, arbutin and isorhamnetin glycosides are characteristic of pear juice and they may provide an index for detection of pear juice in apple juice concentrates. On the other hand, phloretin glycosides are characteristic of apple

juice and their detection in pear juice can be used as an indicator of adulteration of pear juice with apple juice.

Effect of Variety, Maturity, Processing, and Storage on the Procyanidins. Table VII shows the influence of processing and storage on the procyanidin composition of pear juice. Procyanidin B2 (dimer of epicatechin), trimer, and tetramer along with epicatechin and catechin were present in pear juice processed with SO_2. SO_2-processed juice also contained low levels of B1 (dimer of epicatechin–catechin) and B4 (dimer of catechin–epicatechin). Pear juice, however, processed without SO_2 from either hard or soft Comice, d'Anjou, or Bartlett fruit contained only trace, if any, amounts of procyanidins. Apparently, PPO activity caused complete degradation of procyanidins in the juices processed without SO_2. The effect of SO_2 on the procyanidins of pear juice seems to be more pronounced than that on the procyanidins of grape or apple juices, as procyanidins are present in grape or apple juice processed without SO_2 (Spanos and Wrolstad, 1990; Spanos et al., 1990).

The sensitivity of procyanidins to oxidation does not allow for compositional comparison between juices pressed from ripe and unripe fruit. Some comparison can be made, however, concerning the composition and the influence of processing and storage on the procyanidins of Comice and d'Anjou pear juices pressed from hard fruit in the presence of SO_2. These juices have similar qualitative profiles, with Comice containing much higher levels of procyanidins than d'Anjou. The effect of HTST on the procyanidins is similar to that on the cinnamics previously discussed. Minor procyanidin loss seems to occur during enzymatic clarification, while fining resulted in no apparent procyanidin changes. Bottling and concentration resulted in procyanidin degradation, which was more pronounced in bottling of d'Anjou pear juice. Storage of concentrates for 9 months caused complete procyanidin oxidation.

Total Phenolics by HPLC and by the Colorimetric Assay. The quantitation of total phenolics by HPLC and by the colorimetric procedure is shown in Table VIII. Although colorimetric quantitation results in much higher levels of total phenolics than the HPLC quantitation, some correlation between the two methods was obtained for the juice extracted from Comice ($r^2 = 0.843$) and d'Anjou pears ($r^2 = 0.734$). No correlation, however, was obtained for the juice from Bartlett fruit ($r^2 = 0.243$). Extensive phenolic degradation in the Bartlett juice does not allow for correlation between the specific HPLC and the nonspecific colorimetric quantitation. The discrepancy in total colorimetric quantitation of phenolics in the stored concentrates results from interference of intermediates and final browning products with the colorimetric assay (Van Buren et al., 1976; Spanos and Wrolstad, 1990; Spanos et al., 1990).

Somers and Ziemelis (1980) reported synergistic interaction between SO_2 and *o*-dihydroxy phenolics in response to Folin–Ciocalteu reagent. According to these authors, the interference depends on the ratio of SO_2 to *o*-dihydroxy phenolics and it becomes very significant as the ratio increases. For example in model solutions with molar ratios of SO_2 to caffeic acid being 1/10, 1/5, 1/2, 1/1, and 2/1, the magnitudes of errors due to the SO_2 artifact were 2.9, 5.9, 14.7, 29.8, and 59.7%, respectively. At a molar ratio of 10/1 the magnitude of error was 298%. In an effort to determine whether such interference was occurring in the quantitation of total phenolics by the colorimetric assay, SO_2-processed juice from unripe d'Anjou fruit was treated with PVPP to remove phenolic compounds that would increase the ratio of SO_2 to *o*-dihydroxy phenolics. HPLC quantitation showed that PVPP treatment at 1, 5, and 10% (w/v) levels resulted in approximately 5, 40, and 85% reduction of total phenolics. Similar total phenolic reductions were indicated by colorimetric quantitation, which allowed for good correlation ($r^2 = 0.902$) between HPLC and colorimetric assay in the PVPP-treated samples. The above data indicate that for the low SO_2 (ca. 30 ppm) content of the processed pear juices there is no significant interference between SO_2 and colorimetric quantitation of phenolics.

CONCLUSION

Variety and maturity showed considerable quantitative differences in the cinnamic composition of pear juice. The levels of phenolics, especially cinnamics and procyanidins, in the finished juices were dependent on the degree of enzymatic oxidation. The majority of the oxidation occurred in the pulp before and during pressing. Oxidation continued until HTST treatment and resulted in significant degradation of cinnamics and total procyanidin loss in juices that were not protected by SO_2. The hydrolytic activity on cinnamics by the clarification enzymes was removed with bottling. Fining showed no apparent effect on the phenolic profile while bottling and concentration resulted in some procyanidin oxidation. Arbutin and isorhamnetin glycosides were less affected by processing and since they are characteristic of pear juice, may be used as indicators of adulteration of apple juice with pear juice. Storage of concentrates resulted in formation of HMF, significant loss of cinnamics, and total degradation of procyanidins.

Although there were no indications of significant synergistic interference of SO_2 and *o*-dihydroxy phenolics in response to the Folin–Ciocalteu reagent, colorimetric measurements on total phenolics are not specific and should be interpreted cautiously as nonphenolic compounds can interfere with the assay.

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Identification of Altered Proteins in Nonfat Dry Milk Powder Prepared from Heat-Treated Skim Milk

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Reversed-phase high-performance liquid chromatography (RP-HPLC) and gel electrophoresis (SDS-PAGE) were used to monitor changes in the protein profiles of pooled herd (Holstein, Ayrshire, and Brown Swiss) nonfat milk (NFM) preheated to 63, 74, and 85 °C before spray-drying and storage for 8 months. Elution profiles of rehydrated nonfat dry milk (NFDM) changed with preheat temperature and storage time. A whey–casein complex comprised of BSA, β-lactoglobulin, α-lactalbumin, and κ- and α_{s2}-caseins formed in NFDM samples preheated to 74 and 85 °C. Some renaturation of whey proteins occurred as a result of storage time. The whey–casein complex is stabilized through disulfide linkage. Examination of isolated insoluble material (2–6% of total protein) indicated that it is composed almost entirely of caseins that appear to be aggregated with lactose.

Nonfat dry milk (NFDM) powders prepared from skim milk processed at different temperatures have different functional properties when used for the manufacture of various products. Physicochemical changes occur due to heat treatment, which affect the rehydration of NFDM powders and the functional behavior of the dispersed powder (Pallansch, 1970). Skim milk can be heated before spray-drying to meet certain product specifications on whey protein denaturation. Preheat treatment also affects the water absorption properties of NFDM powders. Low- and medium-heat NFDM (63, 74 °C) are frequently used to make ice cream and dairy beverages, in which some water absorption is necessary. High-heat NFDM (85 °C) absorbs more water, which improves its emulsion stabil-