Phenolic Composition of Kiwifruit Juice

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Phenolic compounds in kiwifruit pulp were separated and characterized by reversed-phase HPLC, and the effect of juice processing on the phenolic composition was studied. Fractionation of phenolic compounds was achieved through selective elution from C-18 cartridges prior to preconcentration and subsequent separation by HPLC. Strongly acidic compounds were identified as derivatives of coumaric and caffeic acids, including chlorogenic acid, protocatechuic acid, and a derivative of 3,4-dihydroxybenzoic acid. The weakly acidic fraction contained epicatechin, catechin, and procyanidins (B3, B2, or B4 and oligomers). Flavonols were present as the glycosides of quercetin (glucoside, rhamnoside, and rutinoside) and kaempferol (rhamnoside and rutinoside). Phenolic compounds were present, at levels of <1−7 mg/L, in clarified juice. The concentration of phenolics was highest after high-temperature short-time treatment (HTST) of juice. Hydrolysis of hydroxycinnamic acids occurred after enzyme addition and HTST treatment. The flavonol glycoside composition is the best identifier of kiwifruit juice.

Keywords: *Phenolic compounds; kiwifruit; juice processing*

INTRODUCTION

The production of high-quality kiwifruit juice is affected by a number of factors including excessive browning, formation of hazes/precipitates, and flavor changes. Extensive studies have been made of the role of ascorbic acid degradation in browning (Wong and Stanton, 1993a,b), the influence of protein composition on haze formation (Dawes et al., 1991, 1994), and the contribution of hexenals to off-flavor production in kiwifruit juice (Young and Paterson, 1985, 1995). Phenolic compounds in fruit are important contributors to the color, flavor, and aging characteristics of fruit products (Lea and Timberlake, 1978; Macheix et al., 1990; Spanos and Wrolstad, 1992; Shahidi and Naczk, 1995). Little published information exists on the phenolic composition of kiwifruit and thus its involvement in product quality changes during processing.

Analyses of total phenolics in kiwifruit indicate the level of phenolic compounds to be low in comparison with other fruit. The total phenolic content of kiwifruit has been reported as 180−220 mg/100 g of fruit 1 month after pollination decreasing to around 100 mg/100 g during growth (Fuke and Matsuoka, 1984). Levels of 38−44 mg /100 mL total phenolics have been reported in kiwifruit juice concentrate, after redilution to 13.5 °Brix (Wong and Stanton, 1993a), and 25−31 mg/100 mL total phenolics have been measured in pressed kiwifruit juice prepared from fruit stored for 7−8 months (Dawes, unpublished). The enzyme polyphenol oxidase has been isolated from kiwifruit, although the low activity and a low level of polyphenol substrates were postulated to be the reason cut surfaces of kiwifruit do not readily brown (Okuse and Ryugo, 1981; Park and Luh, 1985). Enzymic browning in the presence of oxygen occurs in kiwifruit juice, although the major mechanism for browning was assigned to acid-catalyzed ascorbic acid degradation, which occurs mainly in kiwifruit juice concentrate (Wong and Stanton, 1993a,b).

Studies on the separation of phenolic compounds in kiwifruit have primarily been carried out by thin-layer chromatography. An investigation of the tannins in kiwifruit by Sephadex G25 and subsequent paper chromatography identified dimers and trimers of epicatechin (Michaud and Ane-Margail, 1977). The ratio of procyanidin to prodelphinidin units was determined as 70:30 for ripe kiwifruit tannin after Sephadex LH-20 separation and analysis by ^{13}C NMR (Foo and Porter, 1981). The flavonoid composition of leaves, stems, and fruit of different *Actinidia* cultivars has been studied in recent years, using thin-layer chromatography, to aid taxonomy studies (Webby, 1990; Webby et al., 1994). More recently methods for the isolation of the flavonoid group from kiwifruit, involving preliminary separation on polyamide columns followed by separation by HPLC, were developed to identify the presence of kiwifruit in fruit products (Mareck et al., 1990).

This research was undertaken to study the different classes of phenolic compounds present in kiwifruit juice. The major objectives of our investigation were (1) to develop a simple and rapid method for the separation, characterization, and quantitation of the strongly acidic and weakly acidic phenolic compounds in kiwifruit using HPLC and (2) to study the changes in phenolic composition which occur during juice processing in order to determine factors which will affect the quality of juice products and to enable identification of kiwifruit juice in products.

MATERIALS AND METHODS

Preparation of Kiwifruit Juice. Kiwifruits (*Actinidia deliciosa* cv. Hayward) picked at 6.2 ± 1.0 °Brix and stored at 1 °C for 3 months were ripened at room temperature to reach an average firmness of 0.97 kg (Effigi penetrometer, 8-mm head) and soluble solids of 14.9 ± 1.0 °Brix. Whole fruits were processed by the method described by Dawes et al. (1994) and Heatherbell et al. (1990). The fruits were milled in a hammer

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mill, and pectolytic enzymes (50 mg/kg, Rohapect D5L, Röhm, Darmstadt, Germany) were added to the mash followed after 2 h by press aid (2% w/w cellulose fibers) prior to pressing in a rack and cloth press. The total pressed juice extraction yield was 73%. The pressed juice was then heat treated at 90−92 °C for 15 s in a tubular heat exchanger and cooled to 40 °C. An additional 150 mg/kg of pectolytic enzymes was then added to produce a pectin-free juice. The juice was then fined with bentonite (2500 mg/L, Volclay, 5% w/w aqueous solution) and after standing for 24 h the supernatant siphoned off, diatomaceous earth (0.2% w/w, Celite, grade 535) added, and the juice filtered through Carlson-Ford filters (grades 5 and 7) in a plate and frame filter press. Concentration of the juice was carried out to 65−70 °Brix (Alfa Laval Centritherm, CT-1B). Samples were taken at each of the processing stages, after pressing, high-temperature short-time (HTST) treatment, and second addition of pectolytic enzyme, clarification with bentonite, and concentration. The samples were stored frozen (−20 °C) until analyzed. Concentrates were rediluted to 13 °Brix before analysis.

Extraction and Fractionation of Phenolic Compounds. Phenolic extracts of juice samples collected during processing were prepared by the addition of ethanol (60 mL) to the juice (30 mL). All samples were filtered (Whatman No. 4) after 1 h to remove precipitated protein. After concentration on a rotary evaporator (40 °C) to remove all the ethanol, the samples were rediluted to 20 mL with distilled water.

Separation of the extracts into strongly acidic and weakly acidic fractions was carried out by adaptation of a method for determination of phenolic compounds in grapes (Jaworski and Lee, 1987; Ritter et al., 1993). The samples (4 mL) were adjusted to pH 7 with concentrated NaOH and passed through a neutral C-18 cartridge (900 mg, Alltech Associates, IL) prepared by preconditioning with equal volumes of methanol followed by water. The weakly acidic phenolic fractions were then eluted with methanol (4 mL). The strongly acidic fractions were obtained by passing a separate 4-mL sample through a neutral preconditioned cartridge, adjusting the eluent to pH 2.0 with concentrated HCl, and passing it through a second C-18 cartridge, this time preconditioned with equal volumes of methanol followed by HCL (0.01 N), and eluting with methanol (4 mL). The above separation procedures, for both strongly acidic and weakly acidic fractions, were repeated five times, and the combined samples (20 mL) were concentrated in a rotary evaporator (40 °C) to a volume of approximately 7 mL. Samples were filtered through 0.45-μm membranes (Millipore, MA) before HPLC analysis.

Standards. Benzoic acids and hydroxycinnamic acids (caffeic acid, chlorogenic acid, *p*-coumaric acid, ferulic acid, gallic acid, *p*-hydroxybenzoic acid, gentisic acid, procatechuic acid, salicyclic acid, syringic acid, vanillic acid), ascorbic acid, citric acid, quinic acid, catechin, epicatechin, and procyanidin B3 were obtained from Sigma Chemicals (St. Louis, MO). Flavonol 3-glycosides (quercetin and kaempferol 3-glucoside, quercetin and kaempferol 3-rutinoside, quercetin and kaempferol 3-rhamnoside) were purchased from Extrasynthese (Genay, France).

HPLC Analysis. Separation was carried out on a Shimadzu class LC-10A HPLC system with class LC-10 software, gradient pump (LC-10AD), autosampler (SIL-10A), and diode-array detector (SPD-M10AV). A spherisorb reversed-phase C-18 (250 × 4.6 mm, 5 μm, Alltech) column was used for separation with a near linear gradient of acetonitrile in water (adjusted to pH 2.5 with concentrated HCL) at room temperature. The mobile phase gradient was adjusted from 3% acetonitrile in water at time 0 min to 40% acetonitrile at 44 min with a flow rate of 1 mL/min. The injection volume for all samples was 20 μL. Simultaneous monitoring was carried out at three wavelengths (4-nm bandwidth): 280 nm (flavan-3-ols, proanthocyanidins, and benzoic acids), 320 nm (hydroxycinnamic acids), and 360 nm (flavonols). The UV spectrum was recorded between 200 and 400 nm.

Concentrations were calculated from external standards. Reproducibility of standards was ±2%. Peak purity, the measurement of the singularity of the peak components, was determined by the peak purity index which compared the up slope, peak top, and down slope of three spectra.

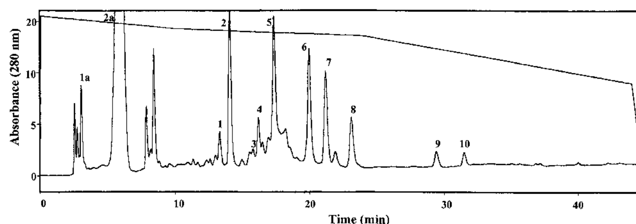

Figure 1. HPLC chromatogram of the strongly acidic phenolic fraction of clarified kiwifruit juice. Peak identifications are given in Table 1.

Table 1. Strongly Acidic Phenolic Compounds Identified in Clarified Kiwifruit Juice (Figures 1 and 2)

peak	retention time (min)	identification
1	13.31	protocatechuic acid
2	14.05	hydroxybenzoic acid or aldehyde, NI[a]
3	15.62	caffeic acid derivative
4	16.21	hydroxybenzoic acid or aldehyde, NI
5	17.35	3,4-dihydroxybenzoic acid derivative
6	19.97	coumaric acid derivative
7	21.20	syringic aldehyde, NI
8	23.10	5-caffeoylquinic acid (chlorogenic acid)
9	29.40	coumaric acid isomer
10	31.47	*p*-coumaric acid

[a]NI, not identified.

RESULTS AND DISCUSSION

Characterization of Strongly Acidic Phenolic Compounds. HPLC chromatograms of the strongly acidic phenolic fraction of clarified kiwifruit juice (after HTST treatment and bentonite fining) are shown in Figure 1. Phenolic compounds were identified by retention times and UV absorbance spectra (diode-array) obtained from standards. The major strongly acidic phenolic compounds separated after an elution time of less than 30 min. Organic acids eluted prior to separation of the strongly acidic phenolics in the increasing gradient of acetonitrile in water. The first two peaks eluting at less than 7-min retention time were identified as ascorbic acid and citric acid, respectively, from standards (peaks 1a and 2a, Figure 1). Kiwifruit juice prepared from *A. deliciosa* has a high titratable acidity of approximately 15.2 g/L (calculated as citric acid), containing citric acid (11.4 g/L), quinic acid (9.7 g/L), malic acid (2.85 g/L), and ascorbic acid (0.72 g/L) (Wong and Stanton, 1993a).

Identification of the hydroxycinnamic acids and hydroxybenzoic acids in clarified kiwifruit juice is represented in Table 1 and Figures 1 and 2. Peak 1 at 13.31-min retention time with absorbance maxima at 260 and 293 nm was identified as protocatechuic acid by comparison with a standard of protocatechuic acid. Peaks 2 and 4, the latter which had a low peak purity in bentonite fined juice, exhibited two UV absorbance maxima, close to 230 nm and between 280 and 300 nm, and were assigned as hydroxybenzoic acids (2-hydroxy or 3-hydroxy) or aldehydes (4-hydroxy) but could not be identified (Bartolomé et al., 1993). Peak 5, which had UV absorbance maxima close to 230, 280, and 310 nm, eluted at a similar retention time to that of the standard of *p*-hydroxybenzoic acid and was assigned as 3,4-dihydroxybenzaldehyde (Bartolomé et al., 1993). Peak 7 at 21.20 min and a small peak, between peaks 7 and 8, observed in HTST juice, eluted at retention times

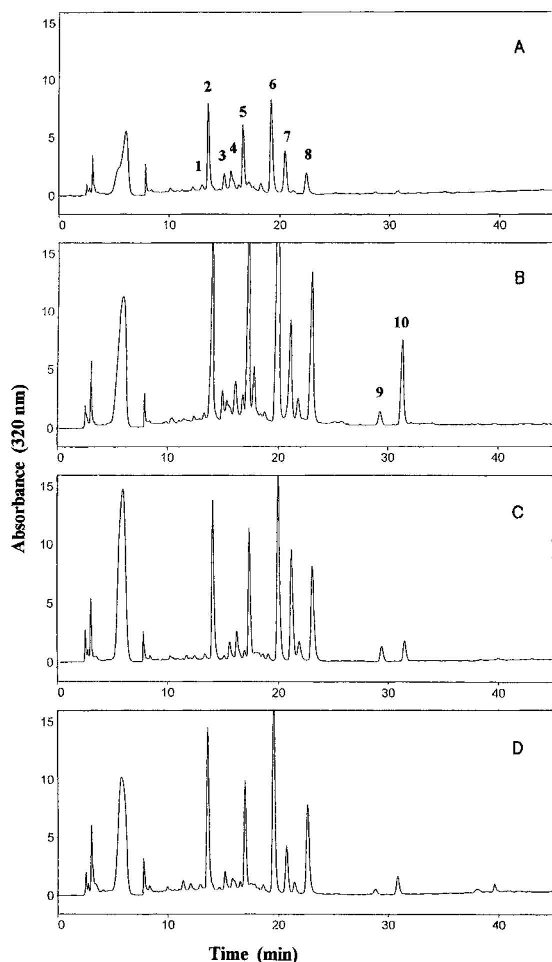

Figure 2. Effect of juice processing on the phenolic composition (strongly acidic fraction) of kiwifruit juice. Detection at 320 nm. Peak identifications are given in Table 1: A, pressed juice; B, HTST (second pectolytic enzyme treatment) juice; C, clarified (bentonite fined) juice; D, concentrated juice (analyzed as rediluted juice, 13 °Brix).

close to that of vanillic and syringic acids. From absorbance spectra peak 7 was tentatively assigned as a syringic aldehyde. Hydroxybenzoic acids in fruit commonly occur as derivatives in the form of glycosides or esters but are also found in the free form in fruit juices (Schuster and Herrmann, 1985; Macheix et al., 1990; Fernández de Simon et al., 1992).

Peak 8 was assigned as 5′-caffeoylquinic acid (chlorogenic acid) based on retention time and UV spectral data of a standard. Low peak purities were obtained for peaks 3 and 6 in pressed kiwifruit juice (0.60 and 0.41, respectively), but from analysis of clarified juice they were identified as hydroxycinnamic acids. Peak 3 exhibited an UV spectrum almost identical to that of a standard of caffeic acid and was assigned as a caffeic or ferulic acid derivative. The UV spectrum shape and maxima for *p*-coumaric acid derivatives is clearly identifiable, but derivatives of caffeic or ferulic acids cannot be differentiated on this basis (Bengoechea et al., 1995). Positional isomers neochlorogenic acid (3′-caffeoylquinic acid) and cryptochlorogenic acid (4′-caffeoylquinic acid) as well as *cis*–*trans* isomers of cinnamic acid occur in other fruit (Spanos et al., 1990). Peak 6 with an absorbance maximum at 309 nm displayed a spectrum identical to that of *p*-coumaric acid and was assigned as a coumaric acid derivative. The UV absorbance

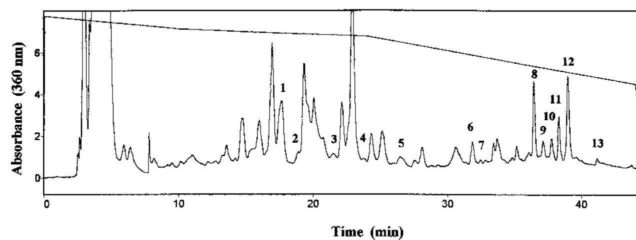

Figure 3. HPLC chromatogram of the weakly acidic phenolic fraction of clarified kiwifruit juice. Peak identifications are given in Table 2.

Table 2. Weakly Acidic Phenolic Compounds Identified in Clarified Kiwifruit Juice (Figure 3)

peak	retention time (min)	identification
1	17.68	contains procyanidin B3
2	18.92 (shoulder of 19.32)	catechin
3	21.47	procyanidin B2 or B4, NI[a]
4	23.30	procyanidin tetramer
5	26.40	epicatechin
6	31.84	procyanidin trimer
7	32.23	procyanidin trimer
8	36.42	quercetin 3-rutinoside
9	37.23	quercetin 3-glucoside
10	37.75	absorbance max 320 nm
11	38.30	kaempferol 3-rutinoside
12	38.96	quercetin 3-rhamnoside
13	41.15	contains kaempferol 3-rhamnoside

[a] NI, not identified.

spectrum of an aglycone esterified with a carboxylic acid or sugar moiety remains substantially unchanged from that of the aglycone; however, retention times are altered due to changes in the polarity of the molecule (Nagels et al., 1979; Moller and Herrmann, 1982; Risch and Herrmann, 1988; Spanos et al., 1990). Hydroxycinnamic acids can be present in the free form but most commonly in fruit as quinic esters or as glycosylated derivatives (Macheix et al., 1990; Fernández de Simon et al., 1992). After HTST treatment and pectolytic enzyme treatment of the juice (Figures 1 and 2), peaks 9 and 10 were detected at significant concentrations. The latter peak from retention data and UV spectrum of standards was assigned as *p*-coumaric acid and peak 9 as an isomer of *p*-coumaric acid.

Characterization of Weakly Acidic Phenolic Compounds. The phenolic fraction eluted with methanol (4 mL) from a neutral preconditioned C-18 cartridge contained weakly acidic phenolics as well as some strongly acidic phenolics. Additional separation by Sephadex LH-20 chromatography as a pretreatment may be necessary to remove interfering compounds that absorb strongly at 280 nm (Spanos and Wrolstad, 1990; Webby, 1990). A representative chromatogram of the weakly acidic phenolic fraction of clarified kiwifruit juice at 360 nm is shown in Figure 3. A total elution time of 42 min was required. The major flavan-3-ols and procyanidins (maximum absorbance close to 280 nm) eluted between 17 and 37 min. Flavonols eluted between 36 and 42 min and were readily identifiable by their absorbance maxima close to 360 nm.

Peaks 2 and 5 were assigned as containing the flavan-3-ols catechin and epicatechin from absorbance spectra and retention times of standards (Table 2 and Figure 3). This was confirmed by the addition of authentic catechin and epicatechin to a clarified juice sample. Catechin (peak 2) eluted at 18.92 min on the shoulder of a peak at 19.32 min. Epicatechin (peak 5) eluted at

Table 3.

(a) Concentration of Strongly Acidic Phenolic Compounds in Kiwifruit Juice during Processing

treatment	protocatechuic	3,4-dihydroxybenzoic derivative	caffeic derivative[b]	coumaric derivative[c]	chlorogenic[b]	*p*-coumaric isomer[c]	*p*-coumaric[c]
	concentration (mg/L)						
	hydroxybenzoic acids[a]		hydroxycinnamic acids				
peak (Table 1)	1	5	3	6	8	9	10
pressed juice	0.26	LP[e]	LP	LP	0.17	ND[e]	0.01
HTST juice	0.24	LP	0.09	1.02	1.11	0.05	0.25
clarified juice	0.24	6.21	0.09	0.52	0.71	0.05	0.06
concd juice[d]	0.27	5.25	0.10	0.55	0.68	0.02	0.06

(b) Concentration of Weakly Acidic Compounds in Kiwifruit Juice during Processing

treatment	catechin	epicatechin	procyanidins[f] tetramer	procyanidins[f] B3	procyanidins[f] B2 or 4	quercetin 3-rutinoside	quercetin 3-glucoside	kaempferol 3-rutinoside	quercetin 3-rhamnoside	kaempferol 3-rhamnoside
	concentration (mg/L)									
	flavan-3-ols					flavonol glycosides				
peak (Table 2)	2	5	4	1	3	8	9	11	12	13
pressed juice	1.14	3.05	ND	ND	4.81	0.25	ND	0.20	0.38	ND
HTST juice	0.90	3.76	2.48	ND	5.52	0.37	ND	0.26	0.43	ND
clarified juice	ND	2.62	ND	ND	4.13	0.41	0.20	0.20	0.45	0.05
concd juice[d]	ND	1.56	ND	ND	4.18	0.44	0.15	0.18	0.39	0.08

[a] Calculated as protocatechuic acid. [b] Calculated as chlorogenic acid. [c] Calculated as *p*-coumaric acid. [d] Concentrated juice (rediluted to 13 °Brix). [e] ND, not determined, low concentration; LP, low peak purity. [f] Calculated as procyanidin B3.

26.40 min. The peak at 17.68 min (peak 1) appeared to contain a procyanidin. A peak purity of 0.80 (in bentonite fined juice) and the UV spectra indicated coeluting compounds at this retention time. Two peaks (3 and 4) eluting at 21.47 and 23.30 min between catechin and epicatechin were similarly assigned as procyanidins from UV spectra. Peak 1 coeluted with the standard procyanidin B3. The distance between the minimum and the maximum in the spectra was calculated as 28 for peak 3 which was assigned as a procyanidin dimer and 22 for peak 4 which was assigned as a tetramer or greater (Bartolomé et al., 1996). Peak 3 was subsequently assigned as procyanidin B4 or procyanidin B2 from the order of elution. Procyanidins in a wine sample were identified as eluting in the order procyanidin B1 < B3 < catechin < B4 < B2, epicatechin using an increasing gradient of acetonitrile in water on reversed-phase HPLC (Jaworski and Lee, 1987; Oszmianski et al., 1988). Procyanidin dimers, trimers, and tetramers in grape seeds were identified by HPLC retention times, diode array spectra, and acid hydrolysis of isolated fractions (Escribano-Bailón et al., 1992). Peak 6 at 31.84 min and peak 7 at 32.23 min (within the 32.4 peak), with UV maxima at 280 nm and values of 25−26 for the distance between the minimum and the maximum in the spectra, were assigned as trimers of catechin/epicatechin from UV spectra (Bartolomé et al., 1996). The major flavan-3-ols present in fruits are epicatechin, which is normally predominant, catechin, gallocatechin, and epigallocatechin, which are present in the free form. In grapes and wine procyanidins are the major proanthocyanidins (Lea et al., 1979; Macheix et al., 1990, Escribano-Bailón et al., 1996).

The flavonols were readily identified by their absorbance at 360 nm. UV spectra were used to differentiate the aglycones, and individual peaks were assigned after comparison of retention times with flavonol glycoside standards. Glycosides of the two flavonol aglycones, quercetin and kaempferol, were shown to be present. Peaks 8 (retention time 36.42 min) and 12 (retention time 38.96 min) which were present at the highest concentration (Table 3b) were identified as quercetin 3-rutinoside (rutin) and quercetin 3-rhamnoside, respectively, from retention times of standards (Table 2). Authentic quercetin 3-rhamnoside and kaempferol 3-glucoside eluted with similar retention times under conditions used. Assignment of peak 12 as quercetin 3-rhamnoside was made on the basis of the UV spectrum, absorbance maxima at 259 and 349 nm (peak purity 0.95 in bentonite fined juice). The remaining peaks were present at lower concentrations. Peak 9 (retention time 37.2 min), which exhibited a UV spectrum similar to that of other quercetin glycosides, had a lower peak purity than other peaks in this region (0.88 in bentonite fined juice) and was assigned as quercetin 3-glucoside from retention data. The third peak in this region (peak 10) was not assigned as a flavonol glycoside. This peak had a low peak purity (0.44 in pressed juice, 0.04 in bentonite fined juice), and absorbance was higher at 320 nm than at 360 nm. Peak 11 was assigned as the diglycoside kaempferol 3-rutinoside from the retention time and UV spectrum of the standard. The small peak at 41-min retention time (peak 13) in bentonite fined juice was tentatively identified as containing kaempferol 3-rhamnoside due to similarity of spectral data and retention time with that of the standard of kaempferol 3-rhamnoside. Using 2D-paper chromatography Webby (1990) described the presence of 13 flavonol 3-glycosides in *Actinidia chinensis* leaf material, while 6 flavonol 3-glycosides were identified in the fruit (quercetin and kaempferol rhamnoside, quercetin and kaempferol rutinoside, quercetin and kaempferol 3-glucoside). Quercetin 3-rhamnoside was found, as in this study, to be the flavonol 3-glycoside present at the highest concentration. In the study by Mareck et al. (1990), involving separation of the flavonol fraction by polyamide columns prior to HPLC separation, 6 glycosides of kaempferol and quercetin were identified. An additional peak, eluting between kaempferol 3-rutinoside and quercetin 3-rhamnoside, was identified by Mareck et al. (1990) as kaempferol 3-galactoside. Kaempferol 3-glucoside was not found. The glycosides of kaempferol, quercetin, myricetin, and isorhamnetin are found in fruits, the most common glycosides are the 3-monoglycosides of

quercetin and kaempferol. The frequency of occurrence of the glycosides in fruit generally decreases in the order: 3-glucosides > 3-rutinosides (diglycoside) > 3-galactosides > 3-rhamnosides > 3-gluronides. The frequencies of the quercetin and kaempferol glycosides are usually similar except for the rhamnosides (Harborne et al., 1975; Markham, 1982; Oleszek et al., 1988; Macheix et al., 1990). The flavonol glycoside of highest concentration in kiwifruit juice, quercetin 3-rhamnoside, has previously only been found in stone fruit, apples, and grapes (Wald and Galensa, 1989; Fernández de Simon et al., 1992; Herrmann, 1994). The flavonol glycoside kaempferol 3-rhamnoside has been identified in bay leaves (*Laurus nobilis L.*) and is only a very minor compound in fruit such as apples (Knackstedt and Herrmann, 1981).

Influence of Juice Processing on Phenolic Composition. Changes in the phenolic composition (strongly acidic fraction) of kiwifruit juice during processing are shown in Figure 2. The concentration of individual phenolic compounds in pressed, heat- and enzyme-treated, bentonite clarified, and concentrated juice is presented in Table 3. Low levels of hydroxycinnamic acids and benzoic acids were found in pressed juice which contrasts with HTST juice which contains the highest levels. Oxidation of phenolic compounds, by the endogenous enzyme polyphenol oxidase, in pressed apple juice prior to pasteurization results in loss of hydroxycinnamic acids (Spanos et al., 1990). Inactivation of polyphenol oxidase after HTST treatment prevents further loss of hydroxycinnamic acids. The HTST juice, which had been treated with pectolytic enzyme, contained considerable levels of free cinnamic acids, in particular *p*-coumaric acid. Concurrently a change in the level of caffeic acid derivatives including chlorogenic acid and coumaric acid derivatives, relative to other peaks in HTST treated juice, from that in pressed juice was also noted (Figure 2, Table 3a). Hydrolysis of cinnamic acid derivatives to the free acid after use of clarification enzymes has been attributed to the presence of esterase activity in commercial enzyme preparations (Spanos et al., 1990). Similarly hydrolysis occurs after HTST treatment. After bentonite fining the concentration of *p*-coumaric acid decreased. Addition of fining agents results in removal of the activity of the clarification enzymes. Reduction in the level of hydroxybenzoic acids also occurred after clarification and concentration. An additional peak at 39.63 min was present after juice concentration, exhibiting a spectrum similar to that of cinnamic acid. The total concentration of the major strongly acidic phenolic compounds in clarified juice is low: hydroxybenzoic acids were present at levels of <7 mg/L and hydroxycinnamic acids at levels of <2 mg/L. The concentrations of the benzoic acids and aldehydes, protocatechuic acid and 3,4-dihydroxybenzoic acid derivatives, were 0.24 and 6.21 mg/L, respectively, in clarified juice. Chlorogenic acid was present at a concentration of 0.71 mg/L in clarified juice. The concentrations of other hydroxybenzoic acids, caffeic acid and coumaric acid derivatives, were 0.09 and 0.52 mg/L, respectively (calculated using chlorogenic acid and *p*-coumaric acid, respectively, as standards, Table 3a). The hydroxybenzoic acid content in fruit is generally low except in certain fruits of the Rosaceae family, in particular blackberry (up to 27 mg/100 g of fresh weight). The amount of hydroxycinnamic derivatives varies over a wide range from minute levels in Cucurbitaceae to a few hundred milligrams per 100 g of fresh weight in blueberries (Macheix et al., 1990).

The highest levels of epicatechin, catechin, and the procyanidins, as for other weakly acidic phenolic compounds, were generally found in the HTST treated juice. Clarification and concentration of the juice to 65 °Brix resulted in some reductions in the concentration of the flavan-3-ols and procyanidins in the latter instance due to oxidation at elevated temperatures (Spanos et al., 1990). The level of epicatechin in clarified juice was approximately 2.62 mg/L, while catechin, which eluted with other unidentified compounds, was present at a lower concentration. Procyanidins were present at a level of up to 4.13 mg/L (Table 3b). The highest reported level of monomeric flavanols in fruit is around 30 mg/100 g of fresh weight; wide variation occurs in the level of hydrolyzable and condensed tannins in fruit (Macheix et al., 1990).

Little change in the concentration of the flavonol glycosides was observed during juice processing. A reduction in the levels in pressed juice compared to HTST treated juice occurred as with the other phenolic classes. The processes of enzyme treatment, clarification, and concentration however resulted in minimal changes. In apple juice the level of flavonol glycosides is least affected by oxidation, whereas other flavonoids and chlorogenic acid are good substrates for polyphenol oxidase (Macheix et al., 1990). The highest concentrations of flavonol glycosides in clarified juice were quercetin 3-rhamnoside at 0.45 mg/L and quercetin 3-rutinoside at 0.41 mg/L (Table 3b). Other flavonol glycosides were present at concentrations of 0.05 mg/L (kaempferol 3-rhamnoside) and 0.20 mg/L (quercetin 3-glucoside and kaempferol 3-rutinoside). Reported levels of flavonol glycosides in fruit range from 0.2 to 30 mg/100 g of fresh weight (Macheix et al., 1990).

CONCLUSIONS

Preliminary separation using C-18 cartridges followed by reversed-phase HPLC provides a rapid method of separation and identification of the phenolic compounds in kiwifruit juice. Overall the level of phenolic compounds in kiwifruit juice is low. The major strongly acidic phenolic compounds in kiwifruit juice have been identified as coumaric and caffeic acid derivatives, including chlorogenic acid, protocatechuic acid, and a derivative of 3,4-dihydroxybenzoic acid. The weakly acidic phenolic compounds in clarified juice include low levels of catechin and epicatechin, procyanidin dimers B3, B2, or B4, and oligomers. The glycosides of quercetin and kaempferol were found, both monoglycosides (quercetin and kaempferol 3-rhamnoside, quercetin 3-glucoside) and the diglycoside (rutinoside).

Pressed juice contains the lowest level of free phenolic compounds. HTST treatment prevented further phenolic oxidation due to polyphenol oxidase inactivation. Clarification with pectolytic enzyme preparations along with HTST treatment resulted in release of coumaric acids from hydroxycinnamic acid derivatives; however, bentonite fining prevented additional hydrolytic enzyme activity. A reduction in the concentration of epicatechin, catechin, procyanidins, and hydroxybenzoic acid derivatives generally occurred after bentonite fining and concentration. The unique flavonol composition, which includes the presence of quercetin and kaempferol 3-rhamnosides, however, is largely unchanged after HTST treatment and is the best identifier of kiwifruit juice.

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