

Phenolic Acid Content and Composition in Leaves and Roots of Common Commercial Sweetpotato (*Ipomea batatas* L.) Cultivars in the United States

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ABSTRACT: Phenolic acids in commercially important sweet potato cultivars grown in the United States were analyzed using reversed-phase high-performance liquid chromatography (HPLC). Caffeic acid, chlorogenic acid, 4,5-di-O-caffeoylquinic acid, 3,5-di-O-caffeoylquinic acid, and 3,4-di-O-caffeoylquinic acid were well separated with an isocratic elution in less than 25 min compared to about 120 min for analyzing and re-equilibrating the column with a gradient method. The isocratic elution order of these caffeoylquinic acid derivatives was confirmed by LC-MS/MS. Chlorogenic acid was the highest in root tissues, while 3,5-di-O-caffeoylquinic acid and/or 4,5-di-O-caffeoylquinic acid were predominant in the leaves. Steam cooking resulted in statistically nonsignificant increases in the concentration of total phenolics and all the individual phenolic acids identified. Sweetpotato leaves had the highest phenolic acid content followed by the peel, whole root, and flesh tissues. However, there was no significant difference in the total phenolic content and antioxidant activity between purees made from the whole and peeled sweet potatoes.

Keywords: caffeoylquinic acid derivatives, liquid chromatography, mass spectrometry, sweet potatoes, total phenolics

Introduction

Recent studies on cultured mammalian cells and animals indicate that polyphenolic compounds from numerous fruits and vegetables exert several health-promoting functions, including reducing the risks of cancer and heart and neurodegenerative diseases (Joseph and others 2005; Vita 2005). Epidemiological studies also show positive associations between intake of fruits and vegetables and reduced mortality rate from heart diseases, common cancers, and other degenerative diseases (Kaur and Kapoor 2001; Art and Hollman 2005; Scalbert and others 2005). The free-radical-scavenging capability and consequent antioxidant properties of the phenolics play an important role in protecting the cells and tissues from oxidative stress and other biological effects associated with these chronic diseases (Rimbach and others 2005).

With such potential health-promoting effects of polyphenols, various studies dealing with the composition and antioxidant activities of phenolics in various fruits and vegetables have been reported in recent years. Several studies on polyphenols in sweet potatoes (*Ipomea batatas* L.) have been carried out, mostly in Japan. The polyphenolic extracts from sweet potato leaves and roots were shown to exhibit high radical-scavenging activity, antimutagenicity, potential chemopreventive properties, and antidiabetic effects

(Oki and others 2002; Suda and others 2003; Rabah and others 2004; Islam 2006; Yoshimoto and others 2006). Islam and others (2002) analyzed the phenolic composition in the leaves of various sweet potato genotypes using gradient high-performance liquid chromatography (HPLC) with 20% to 70% methanol and a run time of more than 60 min per sample. Six phenolic acids in sweet potato leaves were isolated and identified by nuclear magnetic resonance (NMR) spectroscopy (Figure 1). Other investigators used a similar HPLC method to analyze the phenolic composition in the root extracts of sweet potato cultivars commonly used in the processing of several Japanese traditional food products (Yoshimoto and others 2004; Tekenaka and others 2006). A new phenolic acid derivative, 4,5-di-O-caffeoylquinic acid, has been recently isolated and identified by HPLC and NMR in the root extracts of a sweet potato cultivar collected in Peru (Dini and others 2006).

In the United States, sweet potato leaves are not utilized as green vegetables, so the phenolic components in the foliage of commercially grown cultivars have not been determined. With high phenolic content and antioxidant activity, sweet potato leaves can be processed into powders that can be used as functional ingredients in food products such as ice cream, juices, tea drinks, and bread (Islam 2006). Knowledge of the phenolic components and antioxidant activity of sweet potato greens may increase the awareness of the food industry as well as consumers in utilizing sweet potato greens for functional foods. For sweet potato roots, several studies have been conducted on the role of phenolic compounds in darkening of the processed products (Walter and others 1979; Thompson 1981). However, limited information is available on the composition and bioactivity of the phenolics in the roots of U.S. sweet potato cultivars. Walter and others (1979) have developed an HPLC procedure for analyzing the phenolic compounds in sweet potato roots of several cultivars. Four major phenolic compounds of the root extracts

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were isocratically eluted by them from a C18 column with a solvent containing 40% methanol and 60% 33 mM phosphate buffer. Based on retention times, these phenolic compounds were tentatively identified as chlorogenic acid and isochlorogenic acid isomers without further identification.

In the past few years, HPLC, coupled with LC-MS, has been widely utilized in isolating and quantifying phenolic compounds, including phenolic acids, in various food commodities (Naczka and Shahidi 2004). However, applications of these techniques in analyzing sweet potato phenolic profiles are limited. In HPLC analysis, the phenolic compounds adsorbed in a column can be eluted by either short-run or long-run times with isocratic or gradient methods, and an evaluation of these HPLC procedures with sweet potato phenolics has not been reported. The gradient elution procedures that have been developed for analyzing the phenolic acids typically require 65 to 90 min to perform a separation (Islam and others 2002), and an additional time of about 15 min for column re-equilibration before the next injection. Also, gradient methods are prone to problems with reproducibility of retention times. Therefore, the objectives of this study were to determine the phenolic composition in sweet potato leaves and roots using isocratic and gradient HPLC systems and LC-MS/MS, and to evaluate the effect of steam cooking on the phenolic constituents. The total phenolic contents from HPLC analysis and a standard colorimetric method were also compared.

Materials and Methods

Chemicals

Caffeic acid, chlorogenic acid (5-O-caffeoylquinic acid), Folin-Ciocalteu (FC) phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), and HPLC-grade methanol were purchased from Sigma-Aldrich (St. Louis, Mo., U.S.A.). A mixture of dicaffeoyl-quinic acids (3,4-diCQA, 3,5-diCQA, and 4,5-diCQA) was a gift from Dr. Bruce D. Whitaker (USDA-ARS, Beltsville Agricultural Research Center, Md., U.S.A.). Water used for HPLC analysis was purified with a deionized water system (Eagle Water Systems of the Triangle Inc., Durham, N.C., U.S.A.). All other chemicals were analytical grade (Fisher Scientific, Suwanee, Ga., U.S.A.).

Sweetpotato cultivars

The roots and leaves of 2 major commercial cultivars (Beauregard, Hernandez) in the United States and a newly released variety (Covington) from the Sweetpotato Breeding Program, North Carolina State Univ. (NCSU) were used. The 3 cultivars had been grown at the

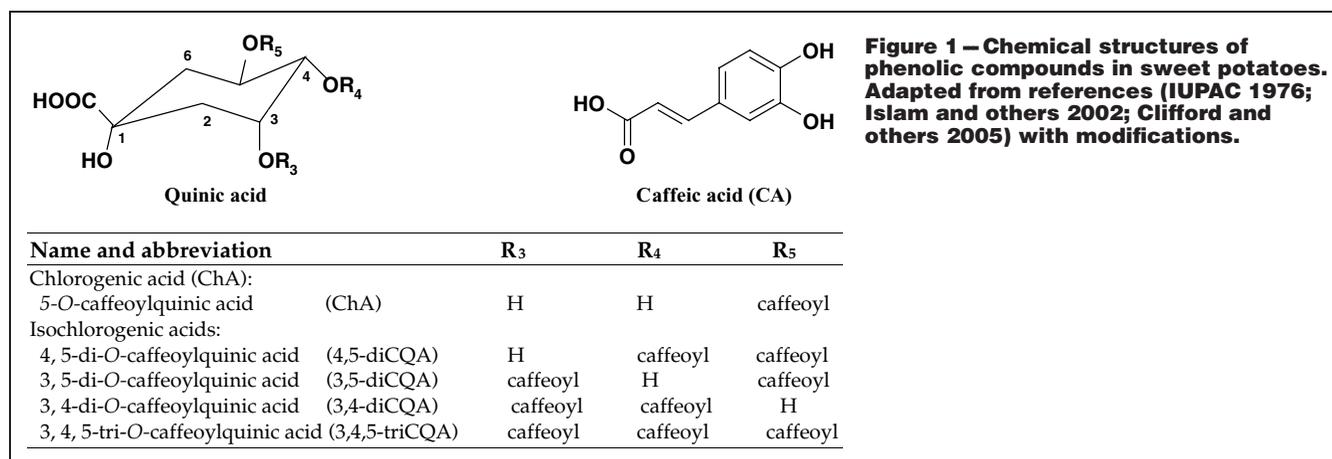
NCSU experimental fields in Clinton and Kinston, N.C., in 2005. The harvested roots were cured at 30 °C, 85% to 90% relative humidity for 7 d, and stored at 13 to 16 °C, 80% to 90% relative humidity for about 1 to 2 wk prior to sampling for analysis. For the sweet potato leaves, the samples were harvested after 120 d of transplanting the cuttings to the fields. Harvested leaves were immediately placed in plastic bags and transported in an ice-cooled container to the laboratory within a day. The leaves were wiped with wet paper towels to remove dirt and then air-dried at room temperature for 15 to 30 min before sampling. Duplicate samples of leaves and roots were collected for each of the 3 cultivars from the 2 experimental fields.

Sample preparation and phenolic extraction

For each sample of the sweet potato roots, at least 10 roots were randomly taken, thoroughly washed with tap water, and air-dried at room temperature overnight. The roots were longitudinally cut and divided into 4 parts. Two parts of the roots, including the peel, were used for whole raw and cooked samples. The remaining samples were peeled with a manual vegetable peeler to obtain peel tissue (1- to 2-mm thickness) and peeled root samples. The samples were designated as peel, raw flesh, and cooked flesh. The collected peel and raw root samples were chopped for 1 min into small particles using a heavy-duty food processor (model RS1 2Y1; Robot Coupe USA, Ridgeland, Miss., U.S.A.). For the cooked samples, the materials were steamed for 45 min in a vegetable steamer and placed in a closed container for cooling to room temperature prior to grinding into puree. The leaf samples (500 g) were also chopped for 1 min with the Robot Coupe food processor. Duplicate specimens from each sample were taken for moisture determination by the oven method at 105 °C and for extraction of phenolic compounds. For phenolic extraction, the samples of leaves and roots (5 to 10 g) were ground in 15 mL boiling 80% ethanol (Walter and others 1979) with a Tekmar tissuemizer (type SDT-1810; Tekmar Co., Cincinnati, Ohio, U.S.A.), and centrifuged at 5000 rpm (Sorvall RC-5B; Du Pont Instruments, Doraville, Ga., U.S.A.) at 5 °C for 10 min. Each sample was extracted 2 more times with 15 mL 80% ethanol and the combined supernatants were brought to 50 mL, filtered with a 0.25- μ m syringe filter, and stored in vacutainers (Becton, Dickinson & Co., Franklin Lakes, N.J., U.S.A.) at -80 °C until analyzed. All the extractions and analyses were performed in a laboratory with UV-filtered light to prevent light degradation of the phenolic compounds.

Colorimetric determination of total phenolics

Total phenolic contents of the extracts were determined by a colorimetric FC method (Singleton and others 1999). Briefly, the filtered



aqueous ethanol extract (0.5 mL) was diluted with distilled water to 5.0 mL to which 0.5 mL FC reagent was added and allowed to react at room temperature for 3 min. One milliliter of 1 N sodium carbonate was added and the mixture was incubated at room temperature for 1 h. The absorbance was measured against a blank at 725 nm using a Cary 300 Bio spectrophotometer (Varian Inc., Research Triangle Park, N.C., U.S.A.). Chlorogenic acid was used as the standard; and the total phenolic content was reported as milligrams of chlorogenic acid equivalents per gram of fresh weight sample (mg ChA/g fw).

Assays of DPPH radical-scavenging activity

Aliquots of the phenolic extracts were diluted (1:10) with 95% ethanol, and the assay was performed following the procedure described by Brand-Williams and others (1995), with minor modifications. The diluted sample, 0.1 mL, was pipetted into 3.9 mL of DPPH solution (0.08 M in 95% ethanol) to initiate the reaction. After a reaction time of 3 h at ambient temperature the reaction had reached completion (Teow 2005), the absorbance was read at 515 nm against ethanol as a blank using a Cary 300 Bio spectrophotometer. Trolox (0, 100, 200, 300, 400, and 500 μ M) was used as a standard. Analysis was done in triplicate for each sample and each concentration of standard. The antioxidant activity was reported in μ moles of Trolox equivalents per gram fresh weight sample (μ mol TE/g fw).

HPLC-DAD analysis and LC-MS/MS identification

The phenolic extracts were analyzed using a Thermo Finnigan HPLC System equipped with a UV6000LP photodiode array detector, AS3000 autosampler, SCM1000 degasser, P2000 binary pump, and ChromQuest software version 4.1 (Thermo Electron Corp., San Jose, Calif., U.S.A.). Separation was achieved on a 250 \times 4.6 mm, 4 μ synergi polar-RP C18 column (Phenomenex, Torrance, Calif., U.S.A.) equipped with a guard column (Allsphere phenyl 5 μ 150 \times 4.6 mm; Alltech, Deerfield, Ill., U.S.A.). The operating conditions were: autosampler sample tray at 6 $^{\circ}$ C; column oven at 35 $^{\circ}$ C; injection volume, 10 to 20 μ L; eluent flow rate, 1 mL/min. The elution solvents were A (0.1% v/v formic acid in water) and B (100% methanol). Spectral data from 200 to 600 nm were recorded and the phenolic chromatograms were monitored at 326 nm. Separation of phenolic compounds by linear gradient and isocratic methods was evaluated. For the linear gradient method, the program started with 2% B from 0 to 15 min, 2% to 10% B from 15 to 20 min, 10% to 45% B from 20 to 90 min, 45% B from 90 to 100 min, and a post-run with 2% B for 10 min to equilibrate the column for the next injection. With the isocratic method, the elution was carried out for 30 min with 60% A and 40% B. Identification of the phenolic compounds was based on retention times and UV spectra with reference to those of commercially available standards such as caffeic acid and chlorogenic acid. For other phenolic compounds, the identification was based on LC-MS/MS analysis, the UV spectra available in the literature, and the retention time of the respective compounds of a mixture of dicaffeoylquinic acids mentioned above (Whitaker and Stommel 2003; Schütz and others 2004). Quantification of all the major phenolic compounds in the HPLC chromatograms was calculated from peak areas with reference to chlorogenic acid standard and expressed as milligrams of chlorogenic acid equivalents per gram of fresh weight of sample (mg CAE/g fw).

LC-MS/MS analyses were performed on an LCS Duo Quadrupole ion-trap mass spectrometer (Thermo Electron, San Jose, Calif., U.S.A.) equipped with an electrospray ionization (ESI) source. The isocratic elution and other HPLC conditions were as described above, except that the flow rate was reduced to 0.4 mL/min. The sheath gas (nitrogen) was set to 65 arbitrary units and the auxiliary gas (He) was set to 10 arbitrary units. The capillary voltage was -26 V

and the temperature was set at 250 $^{\circ}$ C. The ESI source was operated in a negative ion mode with a voltage of -4.5 kV. The instrument was tuned using chlorogenic acid. The collision-induced dissociation was performed in the octapole region at a relative energy of 30%. The data were scanned in a range of 100 to 1000 amu using Xcalibur software (version 1.3, Thermo Electron Co., San Jose, Calif., U.S.A.).

Statistical analysis

The experiment was conducted with 2 replicates in a randomized complete block design. Group differences were evaluated using *t*-tests with *P* < 0.05 considered to be a statistically significant difference. Means were compared with Duncan's multiple range test with α = 0.05 and Pearson correlations were performed using SAS (v. 8.1; SAS Inst. Inc., Cary, N.C., U.S.A.).

Results and Discussion

Total phenolics content and antioxidant activity

The total phenolics content determined by the FC method was not significantly different (*P* > 0.05) among the 3 cultivars, except for the peel samples of the Beaugard cultivar (Table 1). Within a cultivar, the differences in total phenolics in the tissues were significant (*P* < 0.05). The total phenolics content of sweet potato leaves was about 8-, 16-, and 18-fold greater than that of the peel tissue, whole roots, and flesh, respectively. The leaf extracts also had very high radical-scavenging activity with an average value of 38.1 μ mol Trolox equivalents per gram of fresh sample (Table 1). On a dry weight basis, the total phenolics content in the leaves of Beaugard, Covington, and Hernandez was 7.3, 6.9, and 6.7 g per 100 g, respectively. According to the classification of sweet potato genotypes with regard to the total phenolics content (Islam and others 2002), these cultivars can be categorized as medium polyphenolic accumulators. The low and high polyphenolic accumulators contained 5 g and > 9 g ChA equivalent per 100 g of dry weight.

Table 1 – Total phenolics content and radical scavenging activity of leaves and root tissues of sweet potato cultivars

Cultivar	Dry matter (%)	Total phenolics mg ChA/100 g fw	DPPH μ M Trolox/g fw
Leaves			
Beaugard	16.8 (0.0) ^c	1223.6 (50.8) ^a	38.2 (2.4) ^a
Covington	17.7 (0.3) ^c	1224.8 (59.2) ^a	37.6 (0.3) ^a
Hernandez	19.4 (0.1) ^b	1298.1 (71.0) ^a	38.6 (0.9) ^a
Roots			
Beaugard			
Peel raw	14.9 (0.3) ^d	181.7 (20.4) ^b	7.1 (0.8) ^b
Flesh raw	20.5 (0.5) ^b	78.6 (1.5) ^{de}	2.0 (0.2) ^{efg}
Flesh cooked	20.5 (0.4) ^b	88.9 (3.6) ^{de}	2.7 (0.1) ^{def}
Whole raw	20.2 (0.2) ^b	90.3 (13.7) ^{de}	2.8 (0.7) ^{de}
Whole cooked	19.7 (0.1) ^b	101.4 (6.9) ^d	3.6 (0.3) ^{cd}
Covington			
Peel raw	17.4 (0.6) ^c	150.1 (10.7) ^c	5.6 (0.6) ^c
Flesh raw	20.9 (0.7) ^{ab}	57.1 (0.9) ^e	1.1 (0.1) ^{fg}
Flesh cooked	20.3 (0.8) ^b	58.4 (6.8) ^e	1.7 (0.1) ^{efg}
Whole raw	20.5 (0.1) ^b	60.4 (2.5) ^e	1.0 (0.2) ^g
Whole cooked	20.3 (0.5) ^b	78.0 (3.2) ^{de}	2.5 (0.2) ^{def}
Hernandez			
Peel raw	14.2 (0.3) ^d	145.1 (9.9) ^c	4.7 (0.6) ^c
Flesh raw	21.8 (0.0) ^a	72.1 (4.4) ^e	1.5 (0.3) ^{fg}
Flesh cooked	21.2 (0.0) ^a	83.6 (0.2) ^{de}	2.2 (0.1) ^{efg}
Whole raw	20.9 (0.1) ^{ab}	80.4 (2.3) ^{de}	1.8 (0.3) ^{efg}
Whole cooked	20.8 (0.0) ^{ab}	97.8 (1.6) ^{de}	2.9 (0.1) ^{de}

Values in parentheses are standard deviations. Superscripts within a column designate statistically significant differences (*P* < 0.05) by Tukey's multiple range test.

The peel samples from the 3 cultivars had total phenolics contents and DPPH values ranging from 145.1 to 181.7 mg ChA equivalents and 4.7 to 7.1 μmol Trolox equivalents per 100 g of fresh weight,

which were over 2 to 3 times greater than the values in the flesh or the whole roots (Table 1). Similar to our previous report (Teow 2005), the DPPH values were highly correlated with the total phenolics contents ($R^2 = 0.970$). The total phenolics contents in the flesh (57.1 to 78.6 mg/100 g fw) of these commercial cultivars were in the range of 3.3 to 94.9 mg/100 g fw in the sweet potato cultivars reported by Walter and others (1979) and Teow (2005). However, they were much lower than the phenolics content of 945 mg/100g fw in red-fleshed sweet potato roots (Cevallos-Casals and Cisneros-Zevallos 2003). The variation can be attributable to phenolics extraction methods, sweet potato genotypes, and growing conditions (Howard and others 2003).

The differences in total phenolics contents were not significant ($P > 0.05$) between the flesh and whole root samples for both raw and cooked roots (Table 1). Even though the peel contained higher phenolics than the flesh per unit of weight, the peel constituted only 10% to 15% of the weight of the whole root. Table 1 also shows that steam cooking caused an increase in total phenolics content of the puree as compared with the corresponding raw sample, but the differences were not statistically significant ($P > 0.05$). Slight increases in the phenolic content of the cooked samples could be attributable to the release of bound phenolics and inactivation of polyphenoloxidase as affected by the heat treatment. In addition, a certain amount of phenolic acids could be degraded by polyphenoloxidase during chopping of the tissues of the raw sweet potato samples. These results indicated that there was no beneficial effect in terms of total phenolics content and antioxidant activity in using whole sweet potato roots for puree processing. The puree obtained from the whole roots of these orange-flesh cultivars had visible peel particles incorporated in the matrix, which lowered the Hunter color values for lightness (L^*), redness (a^*), and yellowness (b^*), as compared to the purees derived from the peeled roots (data not shown).

Identification of phenolics by HPLC and LC-MS/MS

A typical gradient HPLC chromatogram of the phenolic compounds from Beauregard leaves is shown in Figure 2a. The extracts from the leaves of Covington and Hernandez cultivars exhibited similar gradient chromatographic profiles (data not shown). Five major peaks were separated with elution times of 40, 42, 79, 81, and 90 min. The elution order of these compounds was in accordance with 5 of the 6 phenolics identified in the gradient HPLC chromatograms of sweet potato leaf extracts from various sweet potato genotypes reported by Islam and others (2002). Based on the HPLC elution times and UV absorbance spectra (Table 2), these compounds were tentatively identified as caffeic acid (peak 1), chlorogenic acid (peak 2), 4,5-diCQA (peak 3), 3,5-diCQA (peak 4), and 3,4-diCQA (peak 5). None of the 3 cultivars analyzed showed a detectable peak for the 6th phenolic compound (3,4,5-triCQA) that was reported in the foliage of various sweet potato genotypes in Japan (Islam and others 2002). As sweet potato leaves are not utilized for food in the United States, there has been no previous report on the phenolic acid composition in the foliage of the sweet potato cultivars commercially grown in this country. Walter and others (1979) and

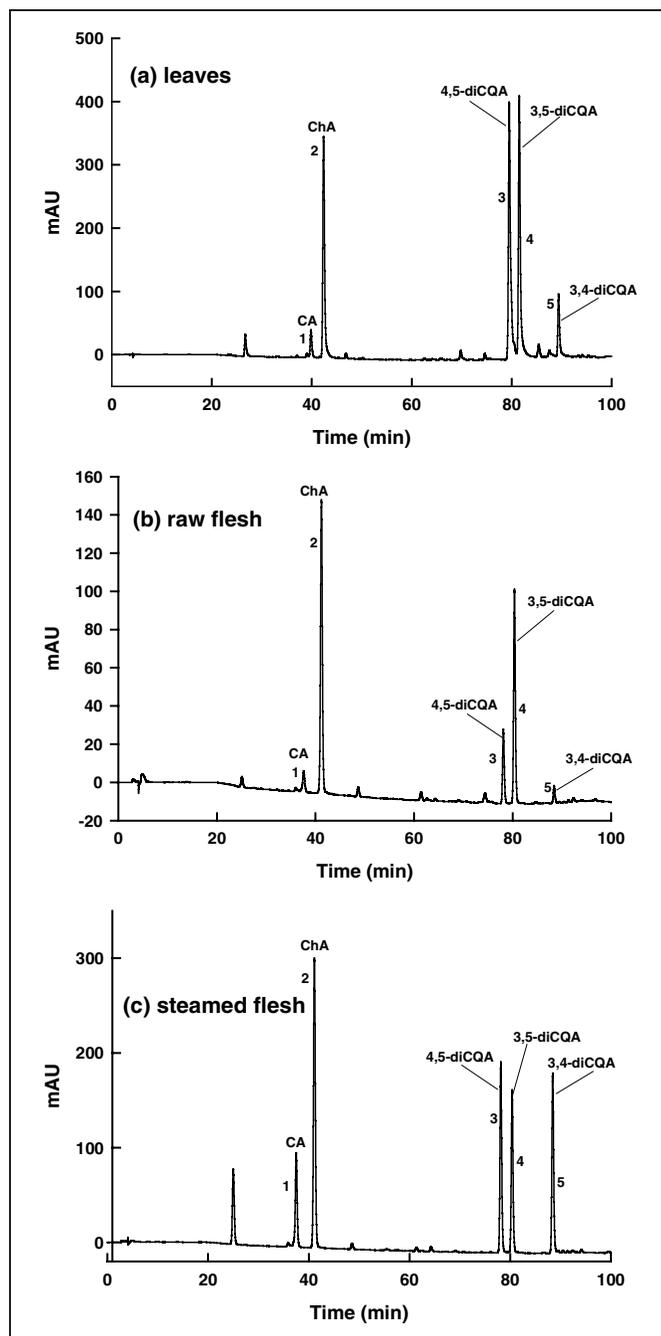


Figure 2—Gradient HPLC chromatograms of phenolic extracts from Beauregard leaves, raw flesh, and cooked flesh

Table 2—Molecular ions, fragmentation pattern, and tentative identification of phenolic compounds in sweet potato leaves and roots by LC-MS/MS

Peak	Compound identity	Retention time* (min)	λ max (230-380 nm)	[M-H] (m/z)	Fragment ion (m/z)
1	ChA	5.03	325, 297 (sh)	353.5	191.3
2	CA	6.27	328, 295	179.3	135.2
3	4,5 di-CQA	11.5	324, 298 (sh)	515.3	352.8, 173.1, 203, 179
4	3,5 di-CQA	12.71	326, 298 (sh)	515.3	352.9, 179
5	3,4 di-CQA	19.72	328, 298 (sh)	515.1	352.9, 173.4, 203

*HPLC with isocratic elution, (sh) = shoulder.

Shimozono and others (1996) did not report the presence of this phenolic compound in the root tissues of sweet potatoes.

The 5 phenolic compounds in the sweet potato leaf extracts were also found to be well separated by isocratic elution (Figure 3a) with shorter retention times: 5.1 (Cha), 6.4 (CA), 12.1 (4,5-di-CQA), 13.3 (3,5-di-CQA), and 20.7 min (3,4-diCQA). All the leaf extracts of the 3 cultivars exhibited an unknown peak at 14.4 min (peak 4b), after peak 4 (3,5-di-CQA). Interestingly, the elution order of caffeic acid and chlorogenic acid of the extracts was reversed as compared to the gradient method. The order of elution by isocratic chromatography was confirmed by the retention time of caffeic acid and chlorogenic acid

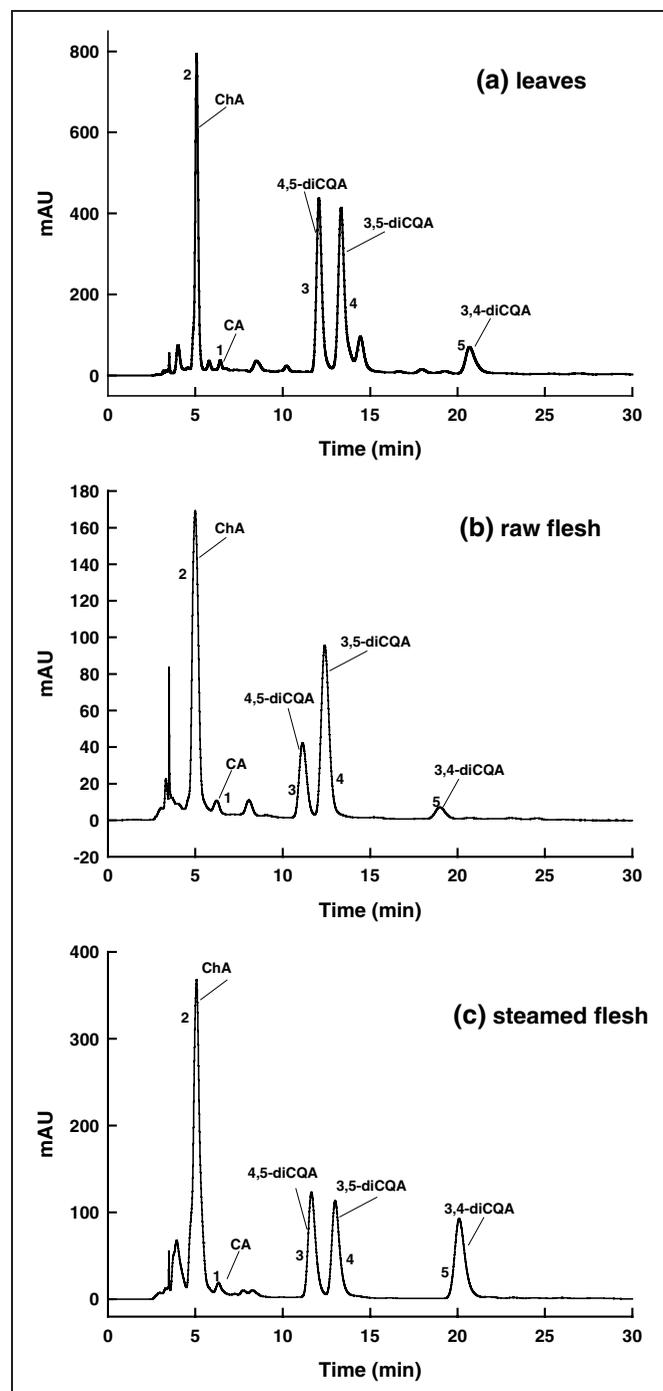


Figure 3—Isocratic HPLC chromatograms of phenolic extracts from Beauregard leaves, raw flesh, and cooked flesh.

standards, their UV spectra, and LC-MS/MS fragment ions (Table 2). A stronger solvent (40% methanol) in isocratic elution as compared to < 20% methanol during the first 45 min of the gradient method and the difference in polarity of caffeic acid and chlorogenic acid affected their retention time. Dalluge and others (1998) also observed a reversal of elution order of (-)-epicatechin and (-)-epigallocatechin gallate in HPLC chromatograms of green tea extracts upon changing the polarity of the solvents by varying methanol–acetonitrile ratios. Wang and others (2000) developed a 60 min isocratic method for analyzing catechins, caffeine, and gallic acid in green tea using a C₁₈ reversed-phase column with a methanol–water–orthophosphoric acid eluant. The responses and relative response factors obtained with the isocratic elution method were very similar to those obtained with a gradient elution system (Wang and others 2003). The isocratic method developed in this study reduces the time per analysis from around 120 min to less than 30 min, achieves near baseline resolution of phenolic acid, and has good reproducibility. This method will allow for rapid, routine analysis of the phenolics in various breeding clones and processed products of sweet potatoes when many samples must be evaluated.

For sweet potato roots, typical HPLC phenolic profiles of the Beauregard extracts eluted with the gradient and isocratic conditions are shown in Figure 2b and 3b (raw flesh), and 2c and 3c (cooked flesh). As shown in these figures, the root extracts also had 5 major phenolic compounds (peaks 1 to 5), which were separated in the same pattern as shown in Figure 2a and 3a for the leaf samples. The extracts from the remaining samples (peel, raw, and cooked whole roots) of all 3 cultivars also showed similar profiles (data not shown). However, the identified compounds had different peak areas, indicating differences in the concentration of these phenolic acids among cultivars and tissues within a cultivar.

Four phenolic compounds were identified as chlorogenic acid and isochlorogenic isomers in the roots of several commercial sweet potato cultivars grown in the United States (Walter and others 1979) and Japan (Shimozono and others 1996). Yoshimoto and others (2004) reported 4 phenolic isomers, namely, ChA, 4,5-diCQA, 3,5-diCQA, and 3,4-diCQA, in a sweet potato cultivar commonly used in liquor processing, and caffeic acid was not found in raw and steamed roots. Recently, Tekenaka and others (2006) reported 6 major phenolic compounds in raw roots of a sweet potato cultivar in Japan. Five caffeic acid derivatives were in accordance with the 5 phenolics, including caffeic acid, isolated in this study (Figure 1). The 6th compound was identified as 6-*O*-caffeoyl- α -D-glucopyranoside (FCG), which was present in high concentration in the root stored for an extended period. This phenolic glycoside was among the compounds that accounted for the high antioxidative activity in sage (Mingfu and others 1999).

Among the 5 phenolic acids in sweet potato leaves and roots only caffeic acid and chlorogenic acid were commercially available as reference compounds for identification. Therefore, HPLC coupled with mass spectrometry was helpful for the identification of individual components when commercial standards were not available. The diagnostic fragmentation patterns by LC-MSⁿ have been reported for chlorogenic acid isomers in dried plums (Fang and others 2002) and green coffee beans (Clifford and others 2005). Phenolic acids ionized well in negative ion electrospray (ESI). In the negative ion ESI spectra of chlorogenic acid, the deprotonated [M-1] molecule of caffeoylquinic acid forms a precursor at *m/z* 353. The ESI MS/MS fragment ions at *m/z* 191 and 173 indicated a quinic acid moiety in the structure, and the ions at *m/z* 179 and 135 were likely derived from a caffeoyl moiety. The relative abundance of fragment ions of peak 3 (*m/z* 353, 173, 203, and 179), peak 4 (*m/z* 353 and 179), and peak 5 (*m/z* 353, 173, and 203) with reference to the [M-1] ion

(*m/z* 515) suggests the identification of these peaks as 4,5-diCQA, 3,5-diCQA, and 3,4-diCQA, respectively (Schütz and others 2004; Clifford and others 2005). The precursor ion and product ion data in Table 2 together with the UV spectrum and the retention time relative to chlorogenic acid confirmed the identification of the 5 phenolic compounds in the HPLC-isocratic chromatograms: peak 1 (caffeic acid), peak 2 (chlorogenic acid), peak 3 (4,5-diCQA), peak 4 (3,5-diCQA), and peak 5 (3,4-diCQA).

Quantification of phenolic compounds by HPLC

The concentrations of individual phenolic compounds were expressed in milligrams of chlorogenic acid equivalents per 100 grams of fresh weight samples since standards for other caffeoylquinic acid derivatives were not available. The quantity of the 5 phenolic compounds in the leaves and different root tissues separated by isocratic HPLC is shown in Table 3. Converting the data of the leaf samples into dry weight basis, the contents of CA, ChA, 4,5-diCQA, 3,5-diCQA, and 3,4-diCQA were, respectively, in the following ranges: 29.2 to 30.7, 683.0 to 814.9, 691.2 to 743.9, 821.3 to 851.8, and 101.7 to 218.8 mg ChAE/100 g of dry weight. These values were within the ranges of individual phenolic compounds in the leaves of 20 sweet potato genotypes reported by Islam and others (2002). Apparently, 3,5-diCQA was the main phenolic compound (32% to 36% of total phenolics) followed by either 4,5-diCQA or ChA (Table 3), which was in accordance with the previous reports (Islam and others 2002; Islam 2006). The concentrations of these 3 phenolic compounds were not significantly different ($P \leq 0.05$) among the leaf samples of the 3 cultivars. In the raw root tissues, the quantities of the identified phenolic compounds in all cultivars were much lower than those of the respective compounds in the leaves. Relative concentrations were in the following order: ChA > 3,5-diCQA > 4,5-diCQA > CA > 3,4-diCQA. Chlorogenic acid constituted about 40% to 60% of the total phenolics in the root samples. Walter and others (1979) also found that chlorogenic acid was the predominant phenolic compound among the compounds they isolated from sweet potato roots of several cultivars. For the puree samples, this same order in the concentration of the individual phenolics was also observed, but

the concentrations of 3,5-diCQA and 4,5-diCQA were very close to each other. Compared to the respective raw flesh and whole root samples, puree processing had a positive effect on the concentration of the isolated phenolic compounds, except for 3,5-diCQA. The changes in the concentration of these isomers can be possibly due to their isomerization by heat and polyphenoloxidase during cooking sweet potatoes as reported by Tekenaka and others (2006).

With the gradient HPLC, the concentrations of the individual phenolic acids in all the sweet potato samples were similar to those of the isocratic method. Overall, the correlation of the concentrations of the corresponding phenolic compounds determined by the isocratic and gradient method was highly significant ($P < 0.001$), except for CA ($R^2 = 0.513$), with ChA ($R^2 = 0.995$), 4,5-diCQA ($R^2 = 0.996$), 3,5-diCQA ($R^2 = 0.999$), and 3,4-diCQA ($R^2 = 0.987$). By summing up the contents of these individual phenolic compounds, the total phenolics measured by HPLC are shown in Table 3. There was a high correlation ($R^2 = 0.995$, $P < 0.001$) between the total

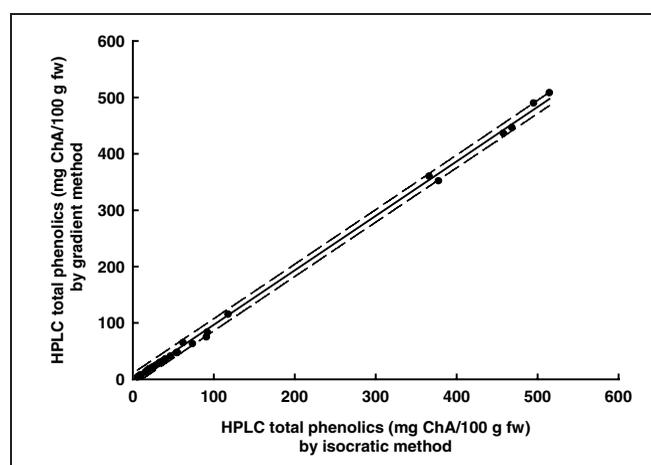


Figure 4—Correlation between the total phenolics content determined by HPLC with gradient and isocratic methods.

Table 3—Individual phenolic compounds in the leaves and root tissues of sweet potato cultivars analyzed by HPLC with isocratic elution

Cultivar/sample	Caffeic acid	Chlorogenic acid	4,5-di-CQA	3,5-di-CQA	3,4-di-CQA	Total phenolics
Leaves						
Beauregard	5.1 (0.1) ^b	126.0 (25.5) ^a	124.9 (21.8) ^a	137.8 (26.7) ^a	36.7 (8.9) ^a	430.5 (82.3) ^a
Covington	5.2 (0.4) ^b	143.8 (28.8) ^a	122.0 (15.1) ^a	145.9 (25.8) ^a	29.1 (15.4) ^{ab}	446.1 (84.5) ^a
Hernandez	6.0 (0.8) ^{ab}	132.8 (13.8) ^a	138.8 (18.9) ^a	165.6 (21.8) ^a	19.8 (4.1) ^b	462.9 (58.8) ^a
Roots						
Beauregard						
Peel raw	6.7 (1.1) ^b	42.8 (6.6) ^b	13.3 (2.2) ^b	38.4 (5.7) ^b	3.6 (1.0) ^{de}	104.8 (15.7) ^b
Flesh raw	1.2 (0.2) ^{efg}	9.3 (0.9) ^{efg}	2.4 (0.4) ^{efg}	6.5 (0.8) ^{ef}	0.5 (0.1) ^g	20.0 (2.2) ^{fg}
Flesh cooked	2.9 (0.6) ^{cd}	16.2 (0.1) ^{def}	5.7 (1.2) ^{cd}	5.4 (0.9) ^{ef}	5.5 (0.9) ^c	35.6 (3.8) ^{efg}
Whole raw	2.2 (1.0) ^{def}	13.6 (3.3) ^{def}	4.3 (1.9) ^{def}	10.7 (3.9) ^e	1.1 (0.6) ^{fg}	31.9 (10.7) ^{efg}
Whole cooked	4.3 (0.8) ^c	21.2 (0.8) ^{cd}	8.5 (1.6) ^c	7.8 (0.9) ^{ef}	8.2 (1.0) ^b	50.2 (5.2) ^{de}
Covington						
Peel raw	3.5 (0.5) ^{cd}	38.8 (5.3) ^b	6.9 (1.1) ^{cd}	31.0 (4.2) ^c	2.0 (0.5) ^f	82.2 (11.4) ^c
Flesh raw	0.3 (0.0) ^g	5.1 (0.5) ^g	0.6 (0.1) ^g	2.3 (0.3) ^f	0.2 (0.0) ^g	8.4 (0.9) ^g
Flesh cooked	1.1 (0.0) ^{efg}	10.6 (1.2) ^{efg}	2.2 (0.0) ^{efg}	2.0 (0.0) ^f	2.4 (0.1) ^{ef}	18.3 (1.3) ^{fg}
Whole raw	0.3 (0.1) ^g	4.6 (1.1) ^g	0.7 (0.2) ^g	2.0 (1.0) ^f	0.1 (0.0) ^g	7.7 (2.4) ^g
Whole cooked	2.3 (0.1) ^{cd}	16.1 (1.0) ^{def}	4.6 (0.1) ^{cd}	4.6 (0.1) ^{ef}	5.3 (0.3) ^c	32.9 (1.6) ^{efg}
Hernandez						
Peel raw	3.4 (0.5) ^{cd}	27.8 (0.7) ^c	6.6 (1.1) ^{cd}	19.7 (2.0) ^d	1.0 (0.0) ^{fg}	58.5 (4.3) ^d
Flesh raw	0.6 (1.6) ^E	6.1 (1.6) ^g	1.2 (0.4) ^{fg}	4.3 (1.4) ^{ef}	0.2 (0.0) ^g	12.4 (3.5) ^g
Flesh cooked	2.1 (0.1) ^{def}	12.5 (1.0) ^{defg}	4.1 (0.2) ^{def}	4.0 (0.2) ^{ef}	4.2 (0.3) ^{cd}	26.9 (1.8) ^{efg}
Whole raw	1.0 (0.2) ^{fg}	7.9 (1.3) ^{fg}	2.1 (0.4) ^g	6.0 (1.3) ^{ef}	0.3 (0.0) ^g	17.3 (3.2) ^{fg}
Whole cooked	3.0 (0.1) ^{cd}	17.1 (0.3) ^{de}	5.9 (0.1) ^{cd}	5.7 (0.1) ^{ef}	5.8 (0.1) ^c	37.6 (0.0) ^{def}

Quantities are in mg ChA/100 g fw. Values in parentheses are standard deviations. Superscripts within a column designate statistically significant differences ($P < 0.05$) by Tukey's multiple range test.

phenolic values obtained from both isocratic and gradient methods (Figure 4).

The total phenolics by HPLC can be directly compared with the results from the FC assay since all the data were expressed in mg ChA equivalents per 100 g fresh weight. The correlation between the 2 methods was highly significant ($R^2 = 0.982$, $P < 0.001$) and the trends among the samples were similar. However, the FC values were about 2-, 3-, and 5-fold higher than the HPLC results of the peel, leaves, and flesh, respectively (Table 1 and 3). Islam and others (2002) analyzed the foliar phenolics in various sweet potato genotypes by HPLC and FC assay. However, their data did not allow a comparison of the 2 methods. The discrepancies between the total phenolics determined by HPLC and FC methods have been reported by several investigators. The FC phenolics were greater than the HPLC values by factors of 10 in red wines (Burns and others 2000), 3 to 6 in champagnes (Chamkha and others 2003), and 4 to 6 in peanuts (Talcott and others 2005). The low total phenolic contents by HPLC can be attributed to the fact that not all the phenolics compounds were isolated, identified, and quantified. On the other hand, the FC reagent can overestimate the total phenolics content, since it also reacts with hydroxyl groups in amino acids and sugars in addition to phenolic groups (Singleton and others 1999).

Conclusions

Major phenolic constituents of sweet potato extracts were separated and quantified using reverse-phase HPLC. A short-run time (under 25 min per sample) can be achieved by isocratic elution with acidified methanol, while gradient elution required up to 120 min for separating the compounds and re-equilibrating the column. Five major phenolic compounds were identified as CA, ChA, 4,5-di-CQA, 3,5-di-CQA, and 3,4-di-CQA. The isocratic elution order of these caffeoylquinic acid derivatives was confirmed by LC-MS/MS. The U.S. commercial cultivars did not contain 3,4,5-triCQA, which has been detected in cultivars grown in Japan, and 4,5-dicaffeoyldaunic acid, which has been recently reported in a sweet potato collection from Peru.

Quantitative analysis of this group of phenolic compounds showed significant variation among the cultivars and tissues. Chlorogenic acid was highest in root tissues, but 3,5-di-CQA and 4,5-di-CQA were predominant in the leaves. Steam cooking resulted in statistically nonsignificant increases in the concentrations of total phenolic components and individual phenolic acids. The FC total phenolic contents in the peel, leaves, and flesh were, respectively, 2-, 3- and 5-fold higher than the sum of individual phenolic compounds isolated by HPLC. Sweetpotato leaves had the highest total phenolic acid content followed by the peel, whole root, and flesh tissues. However, there was no significant difference in the total phenolic content and antioxidant activity between purees made from the whole and peeled sweet potatoes.

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