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Phenolic composition and antioxidant activity of sweetpotatoes [Ipomoea batatas (L.) Lam]

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PHENOLIC COMPOSITION AND ANTIOXIDANT ACTIVITY OF SWEETPOTATOES [*IPOMOEA BATATAS* (L.) LAM]

A Dissertation

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ABSTRACT

The influence of numerous factors on sweetpotato phenolic content and antioxidant activity was determined. Simplified, robust, and rapid methodologies were developed to quantify total phenolics and individual phenolic acids in sweetpotatoes. Quantification of total phenolic content using Folin-Denis reagent provided more reliable results than Folin-Ciocalteu reagent. Individual phenolic acids were quantified by reversed-phase high performance liquid chromatography (HPLC) and the best separation was achieved using a 5-μm, 4.6 × 250 mm column with a mobile phase of 1% (v/v) formic acid aqueous solution: acetonitrile: 2-propanol (70:22:8), pH 2.5. Methanol and ethanol provided higher phenolic extraction efficiency than acetone. In general, chlorogenic and 3,5-dicaffeoylquinic acid were the most prominent phenolics acids in sweetpotato root and leaf tissues. Immature roots and leaves at the initial stages of growth had the highest concentration of phenolics and antioxidant activity. In a comparison of plant parts, sweetpotato leaves had a significantly higher phenolic content and antioxidant activity than roots. Thermal processing of sweetpotato storage roots resulted in a significant loss of phenolic content and antioxidant activity. The outer skin tissue (raw or processed) contained the highest amounts of phenolic acids but also exhibited higher losses than cortex or pith tissue. After a 4 week exposure to 5 °C, the rate of increase in phenolic content and antioxidant activity in non-cured sweepotatoes was significantly higher than in cured roots. An ambient temperature exposure following low temperature storage accentuated the increase in phenolics and antioxidant activity. Minimally processed sweetpotatoes held at 5 °C accumulated more phenolic compounds and had a higher antioxidant activity than sweetpotatoes held at 0 °C. The increase in total phenolic

content and antioxidant activity after 8 days was higher than 4 days. No fresh-cut tissue browning was observed after 8 days and the products were considered to be marketable. Sweetpotato genotypes differ in their phenolic content and antioxidant activity. A purple-fleshed breeding line was found to have higher total phenolic content and antioxidant activity than orange-fleshed and white-fleshed cultivars.

CHAPTER 1. INTRODUCTION

Sweetpotato [*Ipomoea batatas* (L.) Lam.] is a dicotyledonous plant, which belongs to the Convolvulaceae family. The origin of sweetpotato is thought to be Central or South America but it is now grown worldwide in tropical and subtropical regions. Sweetpotato plants produce underground storage roots, which are typically ready to harvest 3-4 months after planting. The freshly harvested roots can potentially be stored for many months using proper postharvest care. Curing of the roots at approximately 32 °C and 90-95% relative humidity for 7 ± 2 days facilitates healing of the wounds incurred during harvest and extends the postharvest life of the roots. Also, curing of sweetpotatoes reduces moisture loss and microbial decay during long-term storage (Picha, 1986).

Based on the production volume, sweetpotato ranks as seventh and fifth most important food crops in the world and developing countries, respectively (CIP, 2006). The sweetpotato can play an important role in solving the global problem of food and energy availability (Kozai et al., 1996). In 2002, the annual per capita consumption of sweetpotato roots was estimated at: China, 43 kg; Japan, 7 kg; U.S., 2 kg; and Europe, 0.1 kg (FAOSTAT, 2005). Despite its high nutritional value, the consumption of sweetpotato is very low in U.S. and Europe. Exploring the health beneficial properties of sweetpotatoes may increase consumer awareness of the positive attributes of this vegetable and help to increase consumption of sweetpotatoes worldwide.

The total world production of sweetpotato is approximately 130 million tons (FAOSTAT, 2005). It is estimated that 98% of the total world sweetpotato production and utilization is in the developing countries (Scott, 1998). Asia is the world's largest sweetpotato-producing region with 125 million metric tons of annual production. China

alone produces 106 million metric tons and contributes almost 90% of worldwide sweetpotato production. In 2005, the annual production of sweetpotatoes in the U.S. was estimated as 714, 000 metric tons with a value at an estimated $ 309 million (NASS, 2005). The sweetpotato is the leading vegetable crop produced in Louisiana, with a production value estimated at $ 46 million (NASS, 2005). Louisiana ranks second in the U.S. after North Carolina in sweetpotato production. The sweetpotato cv. Beauregard is the leading cultivar grown in Louisiana and other parts of the U.S.

NUTRITIONAL IMPORTANCE

Sweetpotato roots are rich sources of crude protein, minerals and carotenoids (Picha, 1985). The high beta carotene content of orange-fleshed roots may help to prevent vitamin A deficiency in developing countries. Besides these components, sweetpotatoes contain phenolic compounds, which may act as antioxidants to safeguard the human body from certain chronic diseases (Hayase and Kato, 1984). In terms of total phenolic content on a fresh weight basis, sweetpotato ranked eighth among 23 most commonly consumed vegetables in the world (Vinson et al., 1998). The term antioxidant is generally used for those compounds that scavenge the free radicals or reactive oxygen species formed in the human body. The human body has a defense mechanism against free radicals, but excessive production of free radicals can cause oxidative damage in cells (Silalahi, 2001). Free radicals impair the biological activity of biomolecules such as DNA, lipids, and protein, causing diseases such as neurodegentrative disorders, aging, cancer, and atherosclerosis (Ames, 1983; Aruoma, 1998). Phenolic compounds may play an important role in preventing chronic illnesses such as cardiovascular diseases, certain type of cancers, neurodegenerative diseases, and diabetes (Surh, 2003; Scalbert et al.,

2005). The antioxidant properties of phenolic compounds have been implicated in suppressing various health related disorders, including melanogenesis in mice (Shimozono et al., 1996), hepatoma invasion (Yagasaki et al., 2000), and human immunodeficiency virus (HIV) replication (Mahmood et al., 1993; Zhu et al., 1999). Recently, Rabah et al. (2004) showed that extracts from baked sweetpotato had potential chemopreventive properties. Juice extracted from purple-fleshed sweetpotatoes had an ameliorative effect against carbon tetrachloride induced liver injury in rats (Suda et al., 1997). Huang et al. (2004) reported that the inhibition of cancer cell proliferation by sweetpotato could be attributed to synergistic effect of phenolics with other phytochemicals.

PHENOLICS IN SWEETPOTATO

Phenolics are compounds having an aromatic ring with one or more hydroxyl groups and functional derivatives (Shahidi and Naczk, 2003). Phenolic compounds are secondary metabolites in plants that are involved in a number of metabolic pathways. They are produced from the aromatic amino acids phenylalanine or tyrosine via the phenylpropanoid pathway. In plants, these metabolites and their derivatives play an important role in cell wall integrity and defense against pathogen attack (Faulds and Williamson, 1999). The two major classes of phenolic compounds include flavonoids and phenolic acids. The biosynthetic pathway for synthesis of the most abundant phenolic acids in sweetpotato (chlorogenic acid and isochlorogenic acid) is shown in Figure 1.1.

Rudkin and Nelson (1947) were the first to isolate phenolic acids from sweetpotato and revealed the presence of chlorogenic acid in the roots. Sweetpotato peelings were reported to contain chlorogenic acid, isochlorogenic acid, caffeic acid,

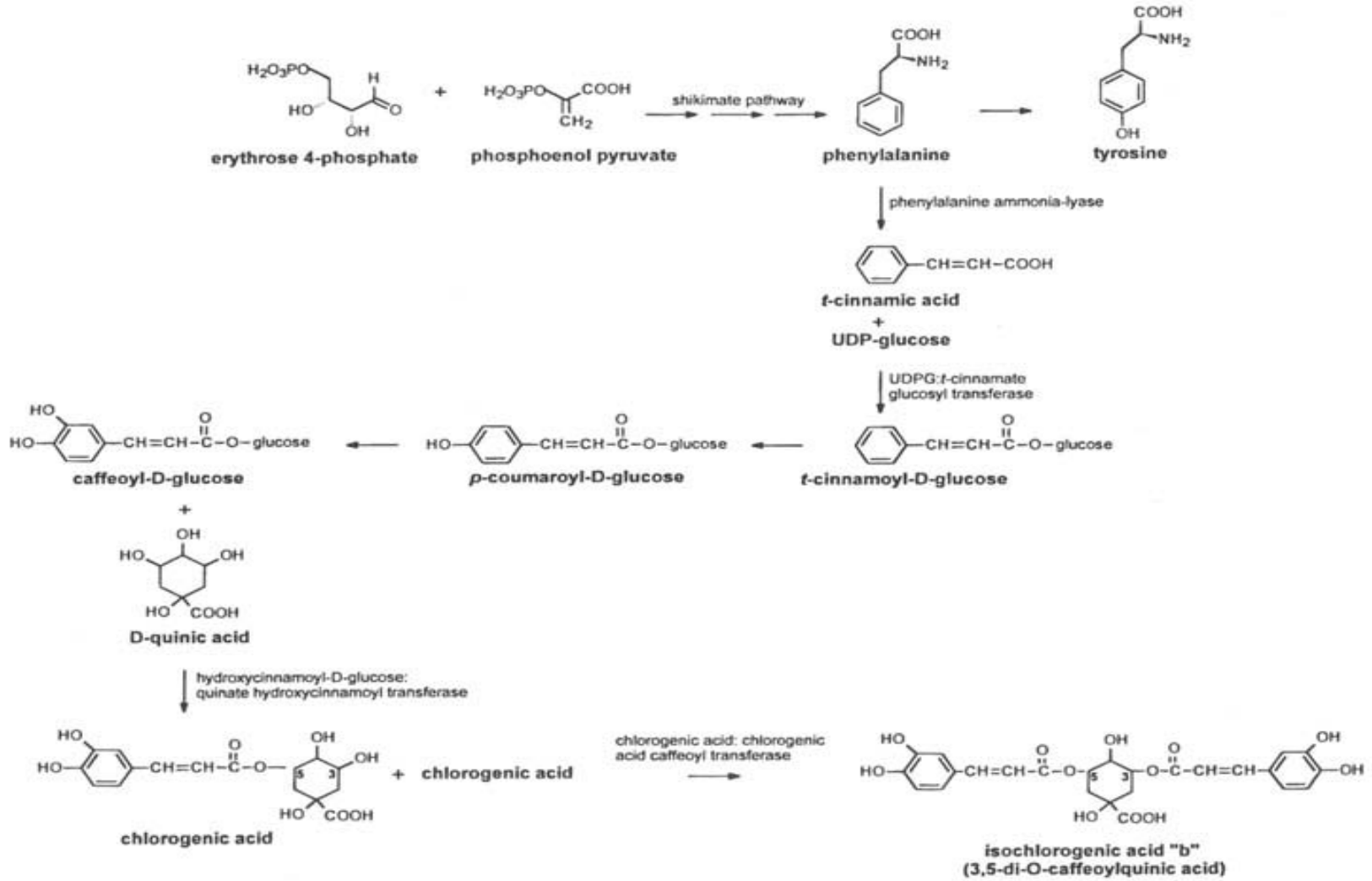
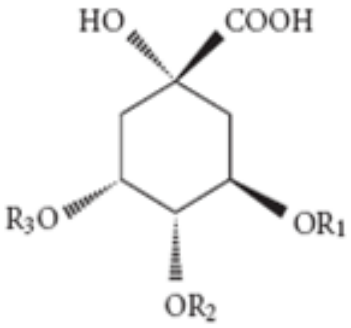


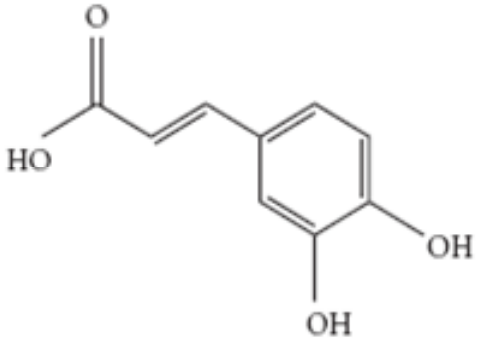
Figure 1.1. Biosynthetic pathways for production of chlorogenic acid and isochlorogenic acid (Friedman, 1997).

neochlorogenic acid, and an unidentified band (Sondheimer, 1958). Walter et al., (1979) were the first to use the high performance liquid chromatography (HPLC) to identify and quantify the individual phenolic acids in different sweetpotato cultivars. In contrast to Sondheimer (1958), caffeic acid was found to be absent in sweetpotato root tissue.

Sweetpotato leaves are consumed as a leafy vegetable in many parts of the world and can be harvested many times during a season (Villareal et al., 1982; Islam et al., 2002). Islam et al. (2002) identified six major phenolic acids in sweetpotato leaf tissue (Figure 1.2.). Previous reports indicated the sweetpotato leaves had a higher antioxidant potential than root tissue (Islam et al., 2003).



Quinic acid



Caffeic acid

Phenolic compound	R_1	R_2	R_3
Chlorogenic acid	Caffeic	H	H
3,5-DCQA	Caffeic	H	Caffeic
3,4-DCQA	Caffeic	Caffeic	H
4,5-DCQA	H	Caffeic	Caffeic
3,4,5-TCQA	Caffeic	Caffeic	Caffeic

Figure 1.2. Molecular structures of phenolic compounds in sweetpotato tissues (cv. Ayamurasaki). diCQA: dicaffeoylquinic acid; triCQA: tricaffeoylquinic acid (Islam et al., 2003).

Despite the health beneficial properties, little is known about the factors that influence the concentration of phenolic compounds and antioxidant activity in sweetpotatoes. This work was initiated to determine the factors affecting the phenolic composition and antioxidant activity in sweetpotatoes. In addition, our goal was to establish simple, rapid, and reliable methodologies to quantify phenolic acids in sweetpotatoes.

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CHAPTER 2. METHOD DEVELOPMENT

INTRODUCTION

Phenolic acids are naturally occurring antioxidant compounds found in different kinds of food materials. Their potential benefits in reducing certain human health related disorders are becoming increasingly apparent. Therefore, simple and robust methodologies for quantifying both total and individual phenolic acids are needed. Due to the heterogenicity of phenolic compounds, several types of reagents have been used for total phenolic content determination in plant extracts. A number of spectrophotometric methods have been developed for quantification of total phenolic content. Folin-type assays (Folin-Denis and Folin-Ciocalteu reagents) are commonly used for quantifying total phenolic compounds in fruits and vegetables. These reagents measure the ability of any mixture to reduce phosphomolybdic and phosphotungstic acids to a blue complex (Swain and Hillis, 1959). The presence of ascorbic acid or other very easily oxidized substances, not considered to be phenolic compounds, may also result in formation of blue color with Folin-Ciocalteu reagent, causing an overestimation of total phenolic content (Shahidi and Naczk, 2003; Singleton et al., 1999). It has been argued that Folin reagents may be inappropriate for plant extracts with high levels of other easily oxidizable substances such as sugars and ascorbic acid (Appel et al., 2001; Escarpa and Gonzalez, 2001). High sugar levels may react appreciably with Folin-Ciocalteu reagent resulting in overestimation of total phenolic content. However, low levels of sugar content may not interfere with total phenolic analysis. Also, the reaction of Folin-Ciocalteu reagent with fructose was shown to be more intense compared to other sugars.

Despite these limitations, Folin-type assays are easy to perform and have been frequently used to estimate the total phenolic content in fruits and vegetables.

Sweetpotato roots are considered to be a good source of phenolic compounds, but also contain fairly high amounts of ascorbic acid and sugars. Folin reagents were previously used for determination of total phenolic content in sweetpotato roots (Cevallos-Casals and Cisneros-Zevallos, 2003; Oki et al., 2002; Walter and Purcell, 1979). However, it has not been determined which reagent (Folin-Denis or Folin-Ciocalteu) is more susceptible to these interfering compounds in sweetpotato roots. The recovery of phenolic compounds from plant material is also influenced by the solvent used and the number of extraction steps (Shahidi and Naczk, 2003). In addition, hexane has been used previously for removing lipids from sweetpotato root tissue (Walter and Purcell, 1979), which could interfere with phenolic assays.

Quantification of both total and individual phenolic acid content is important in sweetpotato nutritional studies. The colorimetric method of measuring total phenolic content does not quantify individual phenolic acids. Reversed-phase HPLC (high performance liquid chromatography) has been used most frequently for analysis of individual phenolic acids (Hayase and Kato, 1984; Son et al., 1991; Walter et al., 1979; Yoshimoto et al., 2004). Many commercially available C18 columns can be used for obtaining good separation of phenolic acids. However, the peak resolution, peak symmetries and retention times may be affected by the difference in column length, bore diameter, particle size and the mobile phase (Ziakova and Brandsteterova, 2003). The most common mobile phases previously used for analysis of phenolic acids in sweetpotato and other crops include methanol, isopropanol, water, acetonitrile, formic

acid and potassium phosphate buffer combinations (Careri et al., 2003; Hayase and Kato, 1984; Son et al., 1991; Walter et al., 1979; Yoshimoto et al., 2004). The use of phosphate buffer resulted in peak broadening and co-elution of some phenolic standards (Tsao and Yang, 2003). Thus, optimization of the mobile phase with a specific column remains an important step in the development of HPLC methodology. Both isocratic and gradient elution has been used for isolating phenolic acids in sweetpotato extracts. A satisfactory resolution could be achieved through isocratic elution by adjusting one or more components of the mobile phase (Robards, 2003). The total analysis time reported for individual phenolic acid quantification in sweetpotato roots ranged from 20-45 minutes (Hayase and Kato, 1984; Son et al., 1991; Walter and Schadel 1981; Yoshimoto et al., 2004). Along with better resolution, a relatively short analysis time is an obvious advantage in quantifying the major phenolic acids in sweetpotato. Despite several studies, a rapid, robust and simple isocratic method for the separation and quantification of individual phenolic acids in sweetpotato roots has yet to be developed.

The objectives of this study were three fold: 1) to determine optimum extraction conditions, 2) to determine the optimal Folin-type reagent to minimize the effect of interfering compounds on quantification of total phenolic content in sweetpotato roots, and 3) to develop a rapid, simple, and robust HPLC methodology using isocratic conditions for individual phenolic acid quantification in sweetpotatoes. C18 columns of different lengths, bore diameters and particle size were compared. The optimal mobile phase was determined after testing various eluent combinations.

MATERIALS AND METHODS

Reagents

Chlorogenic and caffeic acid standards, and Folin reagents (Folin-Denis and Folin-Ciocalteu reagents) were purchased from Sigma-Aldrich (St. Louis, MO). Standards of three isomers (4,5-dicaffeoylquinic acid; 3,5-dicaffeoylquinic acid; and 3,4-dicaffeoylquinic acid) of dicaffeoylquinic acid were kindly provided by Dr. Makoto Yoshimoto (Department of Upland Farming, Kyushu National Agricultural Experiment Station, Miyakonojo, Miyazaki, Japan). HPLC grade solvents were used for elution of individual phenolic acids.

Plant Material

Sweetpotatoes [*Ipomoea batatas* (L.) Lam.] cv. Beauregard roots were harvested in early October, 2004 from the LSU AgCenter Sweetpotato Research Station in Chase, LA. They were immediately cured after harvest at 30 °C and 90% relative humidity for 7 days and then stored at 15 °C until analyzed. Randomly selected sound roots, approximately 300 g in weight, were obtained from storage, washed with tap water and hand peeled prior to tissue extraction.

Sample Preparation and Tissue Extraction

The peeled roots were blended using a Cuisinart Model DLC-2AR food processor (Cuisinart Inc., Windsor, NJ). The blended tissue was passed through a 1 mm sieve. Exactly 1 g of sieved tissue was placed in 15 ml Falcon centrifuge tube and approximately 8 ml of the extraction solvent (80% solution of methanol, ethanol or acetone) was added to the tube. The tubes were capped and immersed in a water bath at 80 °C for 10 minutes. After vigorously shaking the heated samples by hand, the tubes

were cooled and centrifuged at 4,500 g for 15 minutes. The final volume of clear supernatant was made to 10 ml and was used for total phenolics and individual phenolic acid analysis. The addition of hexane to the supernatant from methanol-extracted samples was tested to remove polar lipids and other interfering compounds. The alcoholic supernatant was evaporated under vacuum at 50 °C and the viscous pellet left in the tube was dissolved in 10 ml of water. This solution was extracted with equal volumes of hexane and the aqueous phase was used for analysis.

Total Phenolics

The effect of potentially interfering substances on quantification of total phenolic content was determined by spectrophotometric analysis using Folin-Denis (Swain and Hillis, 1959) and Folin-Ciocalteu assays (Singleton and Rossi, 1965), with slight modifications. Standard solutions of the potentially interfering compounds were prepared in high concentrations (0.05% ascorbic acid, 4% sucrose, 2% fructose, 2% glucose and 10% maltose) previously reported in sweetpotatoes. All solutions were prepared in 80% methanol and also contained 100 µg/ml of chlorogenic acid. Exactly 0.5 ml of standard solution was placed in a 25 ml test tube and mixed with 8 ml of Megapure water (Barnstead MP-12A, Haverhill, MA) followed by the addition of 0.5 ml of Folin-Denis reagent. After 3 minutes, 1 ml of 1 N Na_2CO_3 was added and the solution was allowed to stand for 2 hours at ambient temperature (~21 °C). Absorbance of the resulting blue complex was then measured at 750 nm using a Beckman DU-65 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA). A solution of 80% methanol added to a test tube was used as the blank and 100 µg/ml of chlorogenic acid was used as the control. To perform the Folin-Ciocalteu assay, the Folin-Denis reagent was replaced with 0.25 N

Folin-Ciocalteu reagent. Two separate standard curves for each Folin-reagent were plotted using a linear range of chlorogenic acid concentrations (50-300 µg/ml). Sweetpotato samples with total phenolic concentrations beyond the linear range were diluted before analyzed. The absorbance measured from each solution was presented in terms of chlorogenic acid using the standard curves. Both Folin-reagents were used separately to compare the total phenolic content in the sweetpotato samples. Total phenolic content was expressed as mg chlorogenic acid equivalent/100 g fresh weight.

Individual Phenolic Acids

An aliquot of the supernatant from the sweetpotato extract was filtered through a 0.45 µm Nylaflo membrane filter paper (Pall Corp., East Hills, NY). Individual phenolic acids were separated with four reversed-phase HPLC columns of different length, bore diameter and particle size. The four C18 columns tested were an Alltima Rocket, 3 µm, 7 × 53 mm (Alltech Assoc. Inc., Deerfield, IL), a NovaPak, 4 µm, 3.9 × 150 mm (Waters Corp., Milford, MA), a Gemini long column, 5 µm, 4.6 × 250 mm (Phenomenex Inc., Torrance, CA) and a Gemini short column, 3 µm, 4.6 × 150 mm (Phenomenex Inc.). The column temperature was maintained at 30 °C. A 20 µl aliquot of sample was injected on to each column using a Waters 717 plus autosampler connected to a Waters 600 pump (Waters Corp., Milford, MA). In addition, three different mobile phases were tested. The first mobile phase consisted of 1% (v/v) formic acid in aqueous solution: acetonitrile: 2-propanol (70:22:8), pH 2.5; the second mobile phase contained acetonitrile: 10 mM K_2HP0_4 (20:80), pH 2.6; and the third mobile phase consisted of methanol: 33 mM phosphate buffer (40:60), pH 3.0. A flow rate of 0.75 ml/min was determined to be best suited for all columns, as it provided optimal separation and reasonable low backpressure.

Peaks were detected with a Waters 2487 dual wavelength UV absorbance detector at 320 nm. The detected peaks were identified and quantified by comparing the retention time with that of known standards.

Statistical Analysis

Statistical analysis was performed using SAS (Statistical Analysis Software, version 9.0, Cary, NC). Analysis of variance using procedure Mixed of SAS was performed and means were separated by Tukey's test ($P<0.05$).

RESULTS AND DISCUSSION

Extraction Solvents

Several commonly used solvents were compared for effectiveness of phenolic acid extraction from sweetpotato tissue. Among the extraction solvents tested, acetone gave lower amounts of total phenolics than either methanol or ethanol (Table 2.1). There was no significant difference in phenolic acid extraction effectiveness between methanol and ethanol. Both methanol and ethanol have hydroxyl groups which may form intramolecular hydrogen bonding with hydroxyl groups present in phenolic compounds and increase their solubility (Silla et al., 2001). Hexane, used previously to remove polar lipids (Walter and Purcell, 1979), did not offer any advantage in removing potential interfering compounds in sweetpotato root tissue (Table 2.1). Increasing the number of tissue extractions did not have a significant affect on the amount of phenolics extracted (Table 2.2).

Total Phenolics

Folin-type assays may overestimate the total phenolic content due to reaction with interfering compounds like sugars and ascorbic acid. Our results indicated the Folin-

Table 2.1. Comparison of different solvents for extraction of total phenolics in sweetpotato root tissue.

Solvent	Total phenolics*
80% Methanol	88.6a
80% Ethanol	88.7a
80% Acetone	77.8b
80% Methanol + Hexane	86.7a

Mean values with the same letters are not significantly different at P<0.05 (Tukey's test).
* Data expressed as mg of chlorogenic acid equivalents per 100 g of fresh weight.

Table 2.2. The effect of number of extraction steps (using 80% methanol as solvent) on recovery of total phenolics in sweetpotato root tissue.

Number of extraction steps	Total phenolics*
1	78.2[a]
2	80.0[a]
3	81.1[a]

Mean values with the same letters are not significantly different at P<0.05 (Tukey's test).
* Data expressed as mg of chlorogenic acid equivalents per 100 g of fresh weight.

Ciocalteu reagent was more sensitive to interfering compounds than the Folin-Denis reagent. This may cause overestimation of total phenolic content in sweetpotatoes with high reducing sugar and ascorbic acid content. The extent to which sugars and ascorbic acid affect the results for total phenolic content in sweetpotato tissue might depend on the concentration of interfering compounds. As a consequence, ascorbic acid and fructose resulted in an overestimation of the total phenolic content measured with Folin-Ciocalteu reagent but not with Folin-Denis reagent (Figure 2.1). The Folin-Ciocalteu assay was less affected by other sugars. However, the Folin-Denis reagent was best suited reagent for quantification of total phenolics in sweetpotato, since it was less affected by interfering compounds such as fructose and ascorbic acid. Our results are consistent with other reports indicating the Folin-Ciocalteu reagent resulted in overestimation of total phenolic content by reacting with other non-phenolic compounds (Escarpa and Gonzalez, 2001; Singleton et al., 1999). However, the Folin-Ciocalteu reagent can still be used for

quantifying phenolics in sweetpotatoes, but with a correction factor for the interferences caused by ascorbic acid and sugars.

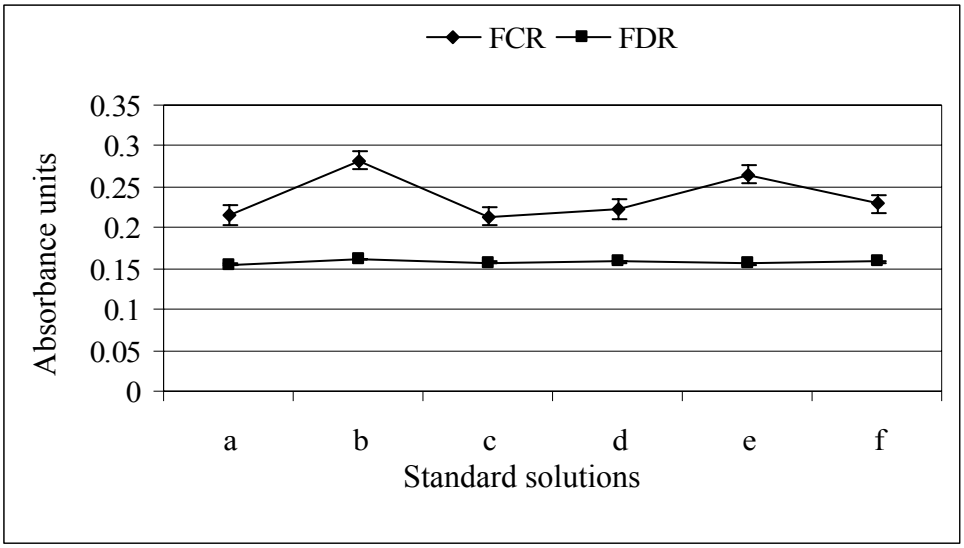

Figure 2.1. Absorbance of standard solutions measured with FDR (Folin-Denis reagent) and FCR (Folin-Ciocalteu reagent). a: 100 µg/ml chlorogenic acid; b: a + 0.05% ascorbic acid; c: a + 4% sucrose; d: a + 2% fructose; e: a + 2% glucose; f: a +10% maltose. Vertical bars represent standard error of the mean (n=5) and, if absent, covered by the symbol.

The average total phenolic acid content in sweetpotato roots, stored at 15 °C for 8 months, was 60.9 mg/100 g fresh weight using the Folin-Denis reagent and 74.6 mg/100 g fresh weight using the Folin-Ciocalteu reagent. The higher amount of total phenolic content with the Folin-Ciocalteu assay may be due to the higher absorbance resulting from reaction with fructose and ascorbic acid.

Individual Phenolic Acids

The optimal mobile phase and flow rate was initially determined for each C18 column. The mobile phase consisting of 1% (v/v) formic acid in aqueous solution: acetonitrile: 2-propanol (70:22:8), pH 2.5 at a flow rate of 0.75 ml/min gave rapid and better baseline resolution of chlorogenic acid (ChlA), caffeic acid (CafA), and three isomers of dicaffeoylquinic acid (diCQA) compared to the other two previously

described mobile phases (Figures 2.2-2.4). Presence of isomers of diCQA in sweetpotato tissue has been documented (Son et al., 1991; Walter and Purcell, 1979). However, the identification and quantification of each isomer of diCQA in sweetpotato cv. Beauregard is reported for the first time.

The four analytical columns were compared using the optimal mobile phase consisting of 1% (v/v) formic acid in aqueous solution: acetonitrile: 2-propanol (70:22:8), pH 2.5. The Gemini C18 columns, irrespective of length and particle size, gave significantly better baseline separation and peak symmetry of individual phenolic acids than the other two columns (Figures 2.4-2.7). However, the total analysis time for individual phenolic acids with the Gemini C18, 3 μm, 4.6 × 150 mm column was 6 minutes compared to 9 minutes in Gemini C18, 5 μm, 4.6 × 250 mm column. A short analysis time helps to save mobile phase and decrease cost in multiple sample analysis. The analysis times are substantially less than the reported run times (20-45 minutes) by other researchers (Hayase and Kato, 1984; Son et al., 1991; Walter and Schadel, 1981; Yoshimoto et al., 2004). An isocratic flow rate of 0.75 ml/min was used for each column, which provides a robust and simplified methodology. No significant interfering impurities were detected and insignificant peak tailing was observed.

The five individual phenolic acids quantified were: ChlA, CafA, 4,5-diCQA, 3,5-diCQA, 3,4-diCQA (Figure 2.4). The phenolic acids profile from all columns indicated chlorogenic acid is the predominant phenolic acid found in sweetpotato roots. The highest peak area for chlorogenic acid was detected using the Novapak C18 column (Figure 2.7). This may be due to co-elution of several small peaks, which are more clearly separated by the Gemini C18 columns (Figures 2.4 and 2.5). The Alltima Rocket

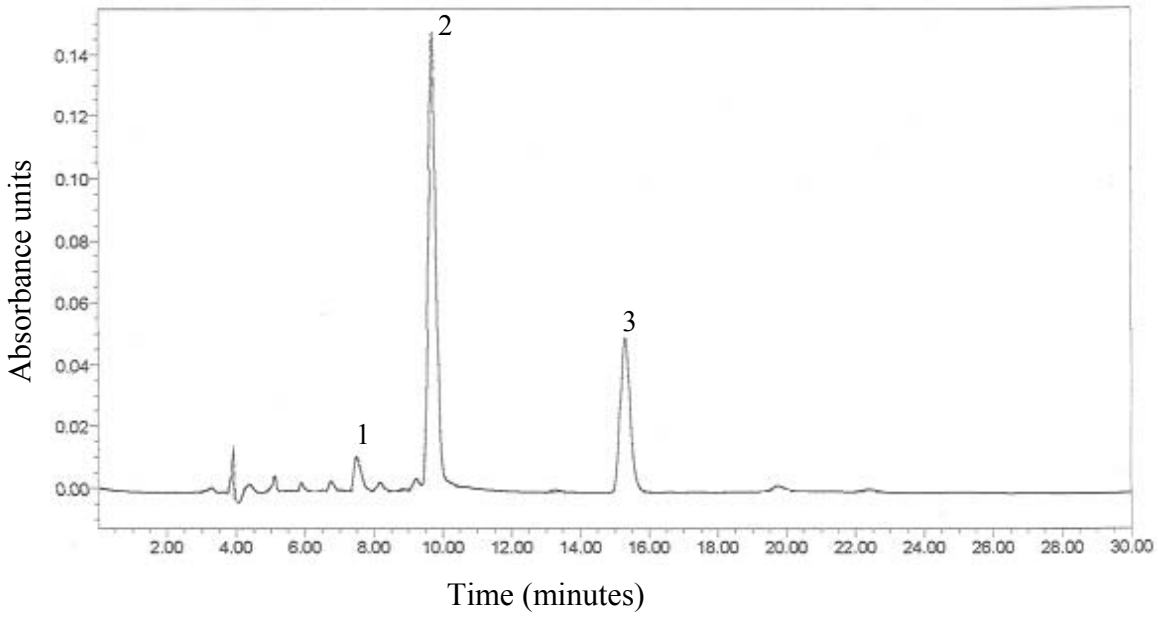

Figure 2.2. A representative chromatogram for a 20 µl sweetpotato extract analyzed with a Gemini C18, 5 µm, 4.6 × 250 mm column using a mobile phase consisting acetonitrile: 10 mM K_2HPO_4 (20:80), pH 2.6; flow rate: 0.75 ml/min, temperature: 30 °C, UV detection at 320 nm. Peaks: (1) unknown, (2) chlorogenic acid, (3) caffeic acid.

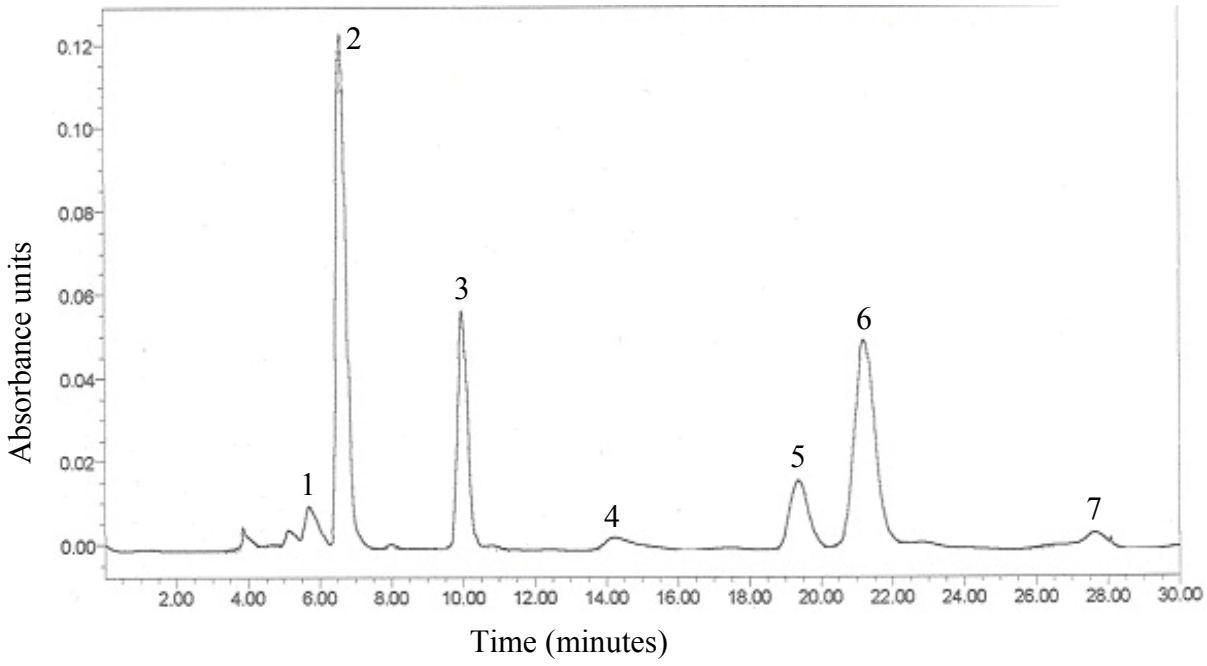

Figure 2.3. A representative chromatogram for a 20 µl sweetpotato extract analyzed with a Gemini C18, 5 µm, 4.6 × 250 mm column using a mobile phase consisting methanol: 33 mM phosphate buffer (40:60), pH 3.0; flow rate: 0.75 ml/min, temperature: 30 °C, UV detection at 320 nm. Peaks: (1&4) unknown, (2) chlorogenic acid, (3) caffeic acid, (5) 4,5-dicaffeoylquinic acid, (6) 3,5-dicaffeoylquinic acid, (7) 3,4-dicaffeoylquinic acid.

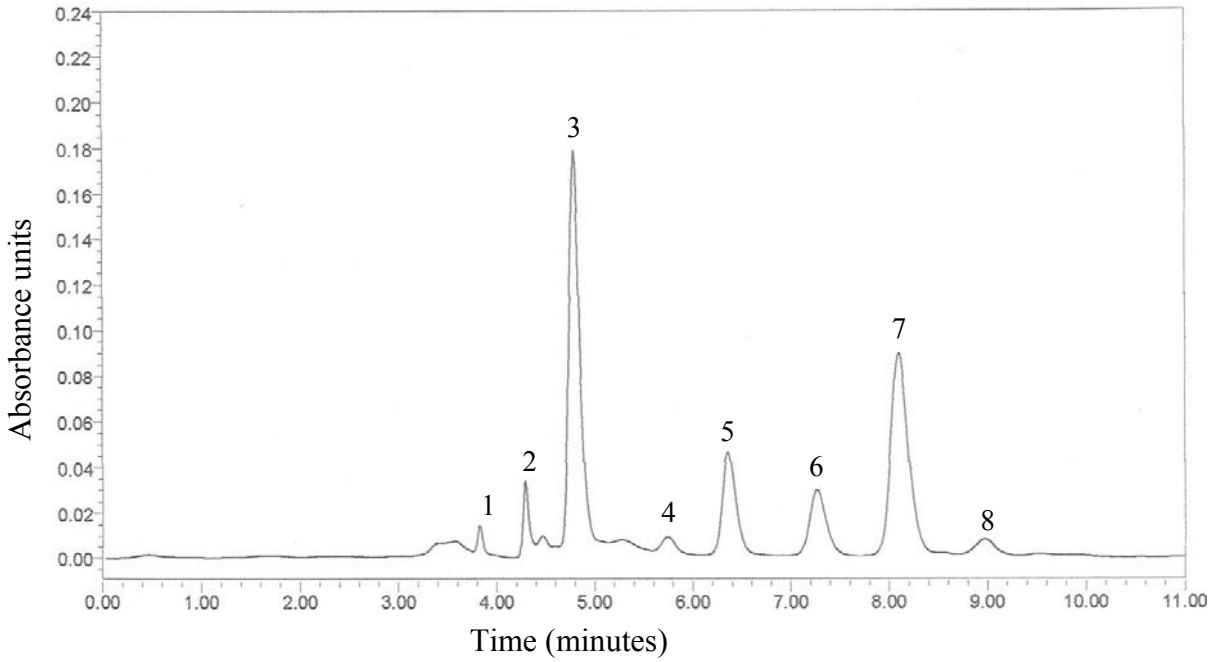

Figure 2.4. A representative chromatogram for a 20 µl sweetpotato extract analyzed with a Gemini C18, 5 µm, 4.6 × 250 mm column using a mobile phase consisting 1% (v/v) formic acid in aqueous solution: acetonitrile: 2-propanol (70:22:8), pH 2.5; flow rate: 0.75 ml/min, temperature: 30 °C, UV detection at 320 nm. Peaks: (1) methanol, (2&4) unknown, (3) chlorogenic acid, (5) caffeic acid, (6) 4,5-dicaffeoylquinic acid, (7) 3,5-dicaffeoylquinic acid, (8) 3,4-dicaffeoylquinic acid.

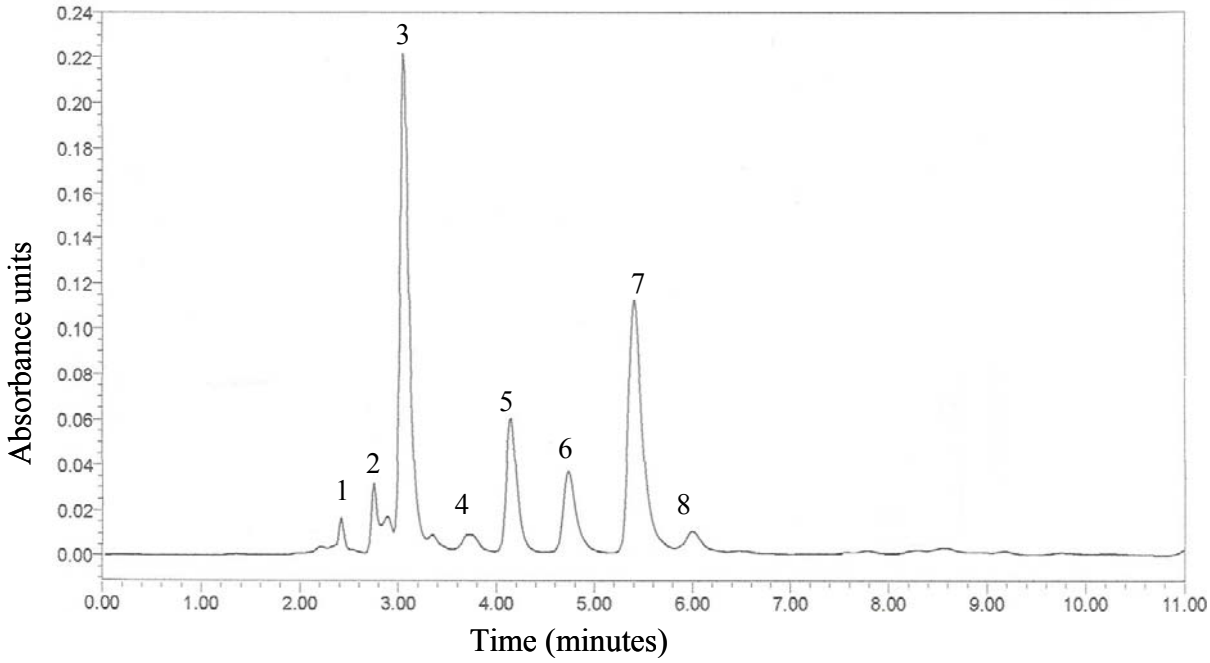

Figure 2.5. A representative chromatogram for a 20 µl sweetpotato extract analyzed with a Gemini C18, 3 µm, 4.6 × 150 mm column using a mobile phase consisting 1% (v/v) formic acid in aqueous solution: acetonitrile: 2-propanol (70:22:8), pH 2.5; flow rate: 0.75 ml/min, temperature: 30 °C, UV detection at 320 nm. Peaks: (1) methanol, (2&4) unknown, (3) chlorogenic acid, (5) caffeic acid, (6) 4,5-dicaffeoylquinic acid, (7) 3,5-dicaffeoylquinic acid, (8) 3,4-dicaffeoylquinic acid.

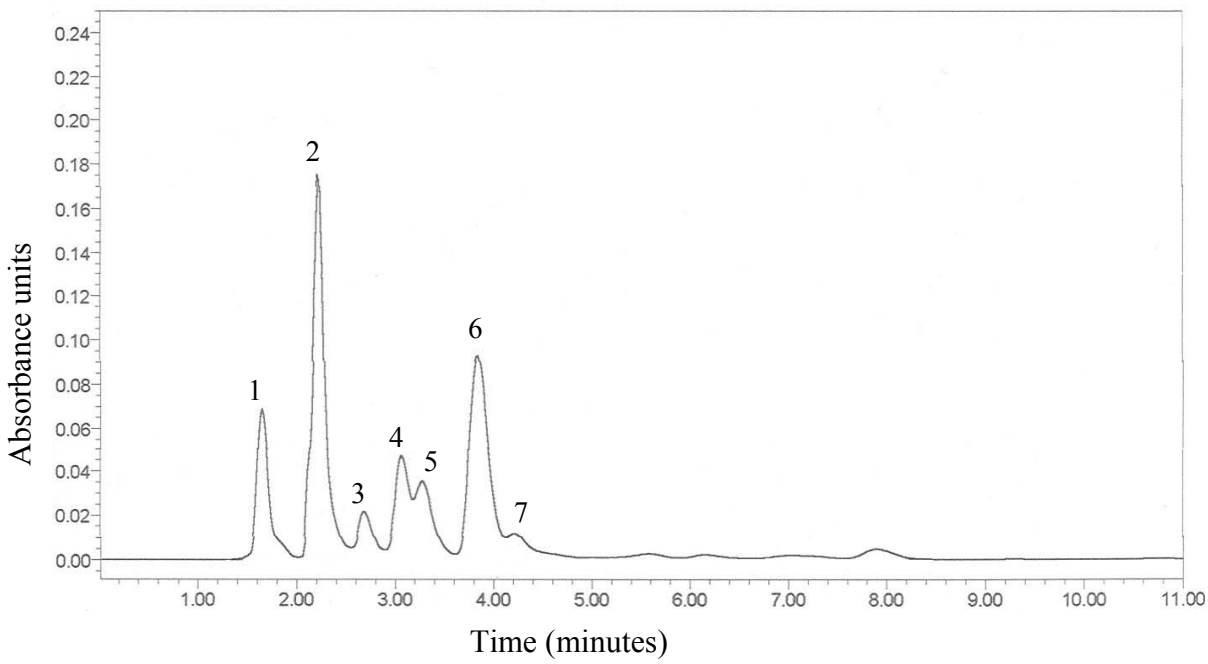

Figure 2.6. A representative chromatogram for a 20 µl sweetpotato extract analyzed with an Alltima C18 Rocket, 3 µm, 7 × 53 mm column using a mobile phase consisting 1% (v/v) formic acid in aqueous solution: acetonitrile: 2-propanol (70:22:8), pH 2.5; flow rate: 0.75 ml/min, temperature: 30 °C, UV detection at 320 nm. Peaks: (1) methanol, (3) unknown, (2) chlorogenic acid, (4) caffeic acid, (5) 4,5-dicaffeoylquinic acid, (6) 3,5-dicaffeoylquinic acid, (7) 3,4-dicaffeoylquinic acid.

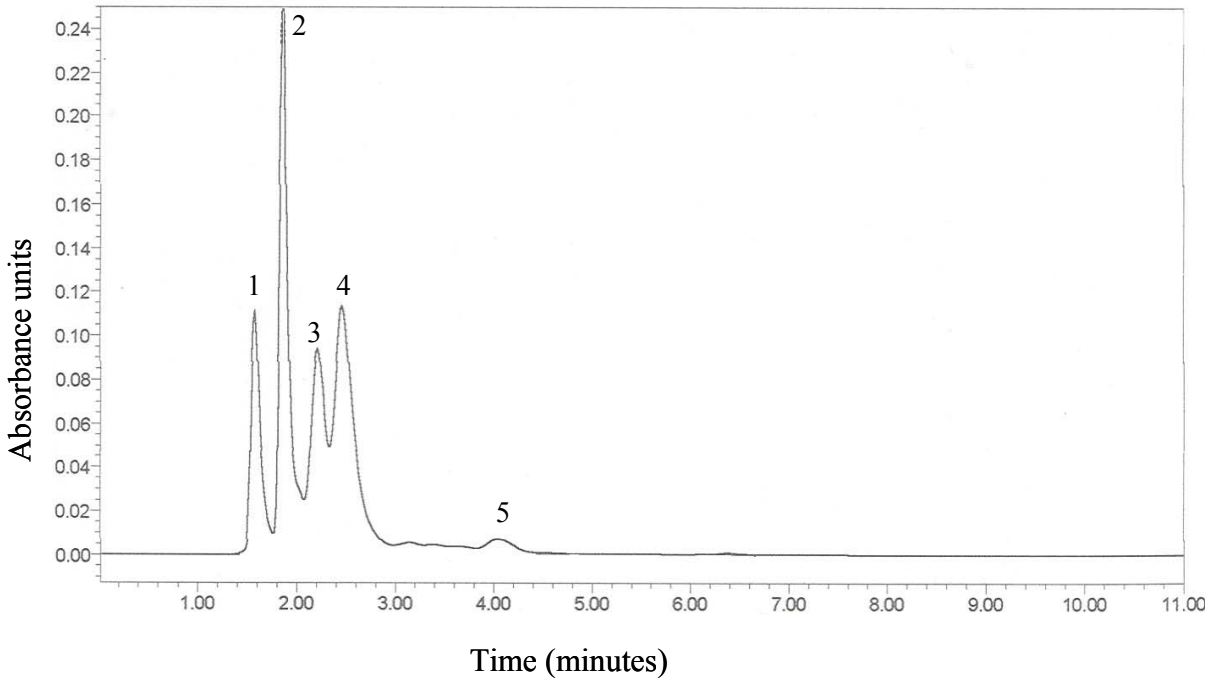

Figure 2.7. A representative HPLC chromatogram for a 20 µl sweetpotato extract analyzed with a Novapak C18, 4 µm, 3.9 × 150 mm column using a mobile phase consisting 1% (v/v) formic acid in aqueous solution: acetonitrile: 2-propanol (70:22:8), pH 2.5; flow rate: 0.75 ml/min, temperature: 30 °C, UV detection at 320 nm. Peaks: (1) methanol, (2) chlorogenic acid, (3) caffeic acid, (4) 3,5-dicaffeoylquinic acid, (5) unknown.

C18 column gave a faster and better separation of phenolic acids and reduced peak tailing compared to the NovaPak C18 column (Figures 2.6 and 2.7). As a consequence, the Alltima Rocket C18 column can be used for separation and quantification of chlorogenic acid and caffeic acid with a short analysis time of under four minutes. Also, the Alltima Rocket column back-pressure was substantially lower (500-600 psi) compared to 1000-1100 psi, 1200-1300 psi, and 2400-2500 psi back pressure in the Novapak, Gemini long, and Gemini short columns, respectively. The low backpressure is an important consideration in the life of a HPLC pump.

The unidentified peaks may be isomers of chlorogenic acid, which need further confirmation. No standards were available for the isomers of chlorogenic acid.

Method Validation

Recovery and precision studies were done for both Gemini columns. Sweetpotato samples were spiked with known amounts of each individual phenolic acid and subjected to the entire extraction procedure. Recoveries for individual phenolic acids ranged from 97% to 102% (Tables 2.3 and 2.4). Precision or repeatability of retention times and peak areas from five standards was calculated using percent relative standard deviation (% RSD) and is presented in Tables 2.3 and 2.4. Relative standard deviation of retention

Table 2.3. Analytical characteristics and method validation data for HPLC using a Gemini 5 µm, 4.6 × 250 mm column.

Phenolic Acid	Linearity range (µg/ml)	r^2	Detection limit (µg/ml)	RSD % (Retention time)	RSD % (Peak area)	Recovery (%)
Chlorogenic	0.1-100	1	0.01	0.12	1.55	101
Caffeic	0.1-50	0.99	0.01	0.11	1.94	102
4,5-diCQA	0.1-100	1	0.01	0.11	1.81	99
3,5-diCQA	0.1-100	1	0.01	0.12	2.13	103
3,4-dCQA	0.1-100	1	0.05	0.10	1.72	98

r^2: coefficient of correlation, RSD %: percent relative standard deviation.

Table 2.4. Analytical characteristics and method validation data for HPLC using a Gemini 3 µm, 4.6 × 150 mm column.

Phenolic Acid	Linear range (µg/ml)	r^2	Detection limit (µg/ml)	RSD % (Retention time)	RSD % (Peak Area)	Recovery (%)
Chlorogenic	0.1-100	1	0.01	0.12	0.94	101
Caffeic	0.1-50	0.99	0.01	0.16	2.93	98
4,5-diCQA	0.1-100	1	0.05	0.29	3.12	100
3,5-diCQA	0.1-100	1	0.05	0.33	1.29	99
3,4-dCQA	0.1-100	1	0.05	0.36	2.84	99

r^2: coefficient of correlation, RSD %: percent relative standard deviation.

times was less than 0.4% and that of peak areas ranged from 0.94 to 3.12%. Linearity of the detector response and limits of detection were tested using different concentrations of each phenolic acid standard. The results are summarized in Tables 2.3 and 2.4.

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CHAPTER 3. ANTIOXIDANT ACTIVITY AND PHENOLIC COMPOSITION CHANGES IN SWEETPOTATO ROOT AND LEAF TISSUE AT DIFFERENT GROWTH STAGES

INTRODUCTION

Numerous epidemiological and biomedical studies indicated the consumption of fruits and vegetables may help to protect the human body against many chronic diseases such as diabetes, cancer and cardiovascular ailments (Ames et al., 1993; Scalbert et al., 2005). This may be due to various naturally occurring nutraceutical compounds present in fresh produce. Phenolics are one class of naturally occurring bioactive compounds present in many fruits and vegetables with nutraceutical properties. Phenolic compounds are antioxidants known to function as singlet and triplet oxygen quenchers and can also scavenge other free radicals. The antioxidant properties of phenolic compounds have been implicated in suppressing various health related disorders, including skin cancer in mice (Kaul and Khanduja, 1998), hepatoma invasion (Yagasaki et al., 2000), and human immunodeficiency virus (HIV) replication (Mahmood et al., 1993; Zhu et al., 1999). The phenolic acids such as chlorogenic acid (ChlA); 3,5-dicaffeoylquinic acid (3,5-diCQA); 3,4-dicaffeoylquinic acid (3,4-diCQA); and 4,5-dicaffeoylquinic acid (4,5-diCQA) extracted from steamed sweetpotato suppressed melanogenesis in mice (Shimozono et al., 1996). The chemopreventive properties of extract from baked sweetpotato have been reported recently (Rabah et al., 2004).

The sweetpotato [*Ipomoea batatas* (L.) Lam.] root is a major staple food widely consumed in developing countries and ranks as the seventh most important food crop in the world (FAOSTAT, 2005). Also, sweetpotato leaves are consumed as a leafy vegetable in many parts of the world (Villareal et al., 1982). Unlike most other

vegetables, sweetpotato leaves can be harvested many times during a season and have a high yield (Islam et al., 2002). In some tropical regions of the world, sweetpotatoes can be grown during the monsoon season and may be the only available green vegetable after a flood or other natural disasters. Both sweetpotato roots and leaves are considered to be rich sources of phenolic compounds (Walter et al., 1979; Islam et al., 2002). Antioxidant activity, antimutagenicity, and free radical scavenging activity of both sweetpotato roots and leaves has been well documented and reported to be higher than other plant materials (Yoshimoto et al., 1999; Yoshimoto et al., 2002; Islam et al., 2003b; Huang et al., 2004). However, these previous studies provided no information on phenolic composition and antioxidant properties of sweetpotato leaves and root tissues at different growth stages. Like other plants, the phenolic content and antioxidant activity of sweetpotato roots and leaves may vary according to the stage of tissue development. A decrease in total phenolic content and antioxidant activity was reported with increased potato tuber weight (Reyes et al., 2004). The objective of this study was to determine the effect of growth stage on phenolic composition and antioxidant activity of sweetpotato root and leaf tissue.

MATERIALS AND METHODS

Reagents

Chlorogenic and caffeic acid standards and Folin-Denis reagent were purchased from Sigma-Aldrich (St. Louis, MO). Standards of three isomers (4,5-dicaffeoylquinic acid; 3,5-dicaffeoylquinic acid; and 3,4-dicaffeoylquinic acid) of isochlorogenic acid were kindly provided by Dr. Makoto Yoshimoto (Department of Upland Farming, Kyushu National Agricultural Experiment Station, Miyakonojo, Miyazaki, Japan).

Plant Material

Sweetpotatoes [*Ipomoea batatas* (L.) Lam.] cv. 'Beauregard' were obtained from plants grown under commercial conditions at the Burden Research Center, Baton Rouge, LA, in the 2005 growing season. Roots were harvested approximately after four months of planting and grouped into 4 different growth stages: I to IV, depending on root weight and size (Table 3.1). Five sound roots from each growth stage were selected for analysis. For leaf analysis, healthy leaf blades were obtained from sweetpotato vines at three different locations: young unfolded leaves from the top of the vine, fully expanded mature leaves from the middle part of the vine, and oldest but fully green leaves near the lower part of the vine. Petioles separated from the old leaves were also analyzed.

Table 3.1. Mean values of weight and size (diameter) of five sweetpotato roots at different growth stages.

Growth stage	Weight (g)	Diameter (cm)
I	4	1.2
II	26	2.6
III	70	3.8
IV	263	6.1

Tissue Preparation

The selected roots were washed and dried at ambient temperature before tissue preparation. The outer periderm layer was gently removed and the retained inner portion was separated into two tissue types: 1) cortex (the tissue external to the cambium layer) and 2) pith (the tissue internal to the cortex). Both root tissue and leaf tissue were immediately frozen at -45 °C, lyophilized, and powdered for further extraction.

Tissue Extraction

Exactly 0.5 g of lyophilized tissue (root or leaf) was placed in a 15 ml Falcon centrifuge tube. Approximately 8 ml of 80% methanol was added to the centrifuge tube.

The tubes were capped and immersed in a water bath at 80 °C for 10 minutes. After vigorously shaking the heated samples manually for 30 seconds, the tubes were cooled and centrifuged at 4,500 g for 15 minutes. The clear supernatant was decanted and brought to a final volume of 10 ml and analyzed for total phenolic content, individual phenolic acids content, and antioxidant activity.

Total Phenolics

Total phenolic content was determined by modification of the Folin-Denis method (Swain and Hillis, 1959). Exactly 0.5 ml of supernatant was placed in a 25 ml test tube and mixed with 8 ml of Megapure water (Barnstead MP-12A, Haverhill, MA) followed by the addition of 0.5 ml of Folin-Denis reagent. After 3 minutes, 1 ml of 1 N Na_2CO_3 was added and the solution was allowed to stand for 2 hours at ambient temperature (~22 °C). Absorbance of the resulting blue complex was measured at 750 nm using a Lambda 35 UV/Vis spectrophotometer (PerkinElmer Instruments, Norwalk, CT). A standard curve of chlorogenic acid (50-300 µg/ml concentration) was plotted. The total phenolic content was expressed as mg ChlA equivalent/g dry weight.

Antioxidant Activity

The antioxidant activity was measured according to method developed by Brand-Williams et al. (1995), with slight modifications. DPPH (1, 1-diphenyl-2-picrylhydazyl) was used as the source of free radicals. The absorbance of free radicals at 520 nm disappears upon their reduction by an antioxidant. In this study, Trolox (6-hydroxy-2, 5, 7, 8-tetramethyl-chroman-2-carboxylic acid) was used as the standard antioxidant compound. An aliquot (300 µl) of sweetpotato extract was placed in a 1.5 ml amber colored centrifuge tube. Then 600 µl of 0.1M 2-(N-morpholine) ethanesulfonic acid

(MES) (pH 6.0) and 300 µl of 0.4 mM of DPPH solution were added to the centrifuge tube. The resultant mixture was shaken and held in the dark for 2 minutes. The decrease in DPPH absorbance was measured at 520 nm using a Lambda 35 UV/Vis spectrophotometer (PerkinElmer Instruments, Norwalk, CT). The sweetpotato extract was replaced with 80% methanol in the control sample. A solution of 80% methanol without DPPH was used as blank. The antioxidant activity was calculated from a standard curve made with known concentrations of Trolox and expressed in terms of mg Trolox equivalent/g dry weight.

Individual Phenolic Acids

Separation of individual phenolic acids was accomplished using reversed-phase HPLC with a Gemini C18, 5 µm, 4.6 × 250mm column (Phenomenex, Torrance, CA). An aliquot of the supernatant was filtered through a 0.45 µm Nylaflo membrane filter (Pall Corp., East Hills, NY). A sample volume of 20 µl was injected onto the column using a Waters 717 autosampler connected to a Waters 600E pump (Waters Corp., Milford, MA). Phenolic acids were eluted using a mobile phase consisting 1% (v/v) formic acid in aqueous solution: acetonitrile: 2-propanol (70:22:8), pH 2.5, with an isocratic flow rate of 0.75 ml/min. Peaks detected were identified and quantified by comparing the retention time and peak area to that of known standards. Quantification was based on absorbance at 320 nm using a Waters 2487 dual wavelength UV absorbance detector.

Statistical Analysis

A completely randomized design was used with five replications. Each root was considered a replication. Data was analyzed by SAS procedure MIXED (SAS Institute Inc., Cary, NC). Analysis of variance using procedure was performed and significance of

differences between means were determined by Tukey's test ($p<0.05$). A Pearson correlation was used to determine the relationship between total phenolics content and antioxidant activity.

RESULTS AND DISCUSSION

Total Phenolics

The small roots at growth stage I had a significantly higher ($P<0.05$) total phenolic content (10.3 mg/g dry weight in cortex and 9.0 mg/g dry weight in pith) than fully developed roots at stage IV (Figure 3.1). Total phenolic content decreased in root tissue as they continued to enlarge. A decrease in concentration of total phenolic content with development of potato tuber was attributed to a dilution effect due to increase in

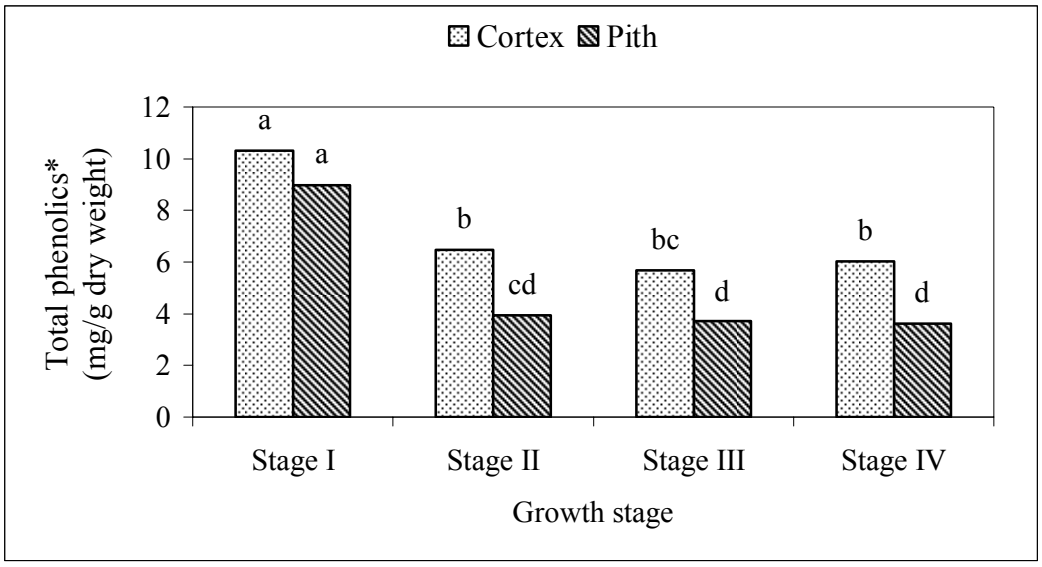

Figure 3.1. Total phenolics in sweetpotato root tissues at different growth stages. Means with different letters are significantly different at $P<0.05$ (Tukey's test).*Data expressed as mg of chlorogenic acid equivalents per g of dry weight.

tuber weight (Reyes et al., 2004). It has been speculated that young roots may have high auxin level facilitating accumulation of more phenolic compounds (Liu et al., 1996). As

reported previously (Walter and Schadel, 1981), cortex tissue had higher total phenolics than the pith tissue, although no significant differences were observed at stage I.

In agreement with Islam et al. (2002), our results indicated sweetpotato leaves had a significantly higher total phenolic content than root tissues. In addition, this study revealed the total phenolic content varies with the growth stage or age of sweetpotato leaves (Figure 3.2). The concentration of phenolic compounds in young leaves was significantly higher than in mature and old leaves. Results from a separate analysis

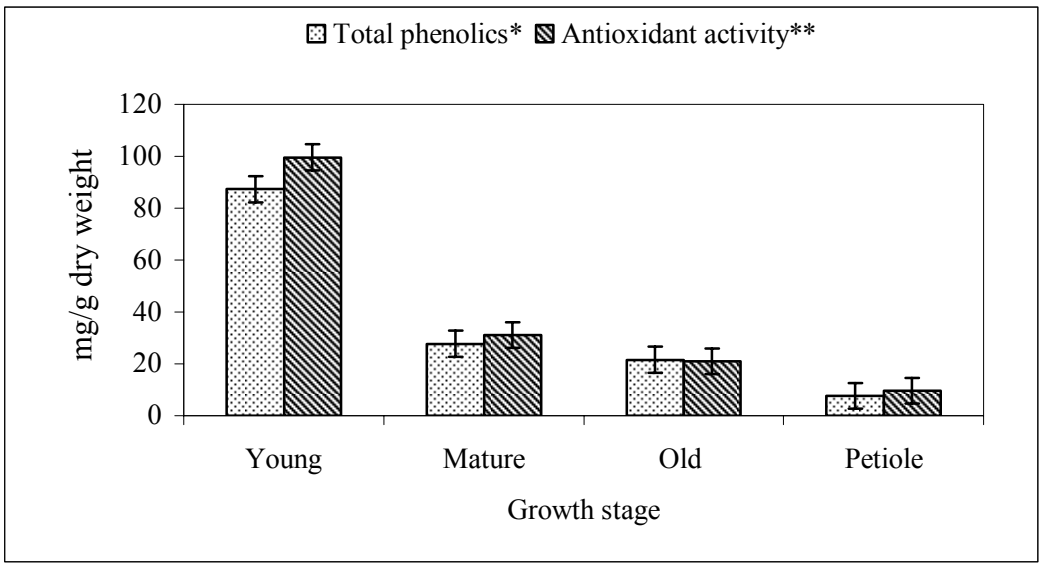

Figure 3.2. Total phenolics and antioxidant activity of sweetpotato leaf tissue at different growth stages. Vertical bars represent standard error of the mean. *Data expressed as mg of chlorogenic acid equivalents per g of dry weight. **Data expressed as mg of Trolox equivalents per g of dry weight.

indicated the leaf blade had a higher total phenolic content than the petiole. Total phenolic content in the young leaf blade (88.5 mg/g dry weight) was approximately 4-fold higher than in the old leaf blade (21.8 mg/g dry weight), which in turn was about 3-fold higher than in the petiole (7.9 mg/g dry weight). Previous reports indicated the young leaves from tea and strawberry had higher total phenolic content than old leaves (Lin et al., 1996; Wang and Lin, 2000). The higher content of phenolic content in young

tissue may be due to higher auxin levels. Studies on other crops indicated the phenolic compounds regulate auxin transport and accumulate in tissues with high auxin production (Faivre-Rampant et al., 2000).

Antioxidant Activity

The changes in antioxidant activity of developing root and leaf tissues of sweetpotato paralleled that of total phenolic content. Roots at growth stage I had significantly higher antioxidant activity (9.7 mg/g dry weight in cortex and 9.2 mg/g dry weight in pith) than roots in the other three stages (Figure 3.3). Similar to the total phenolic content, the antioxidant activity of sweetpotato roots decreased with the increase in root size. Cortex tissue always had higher antioxidant activity than the pith tissue,

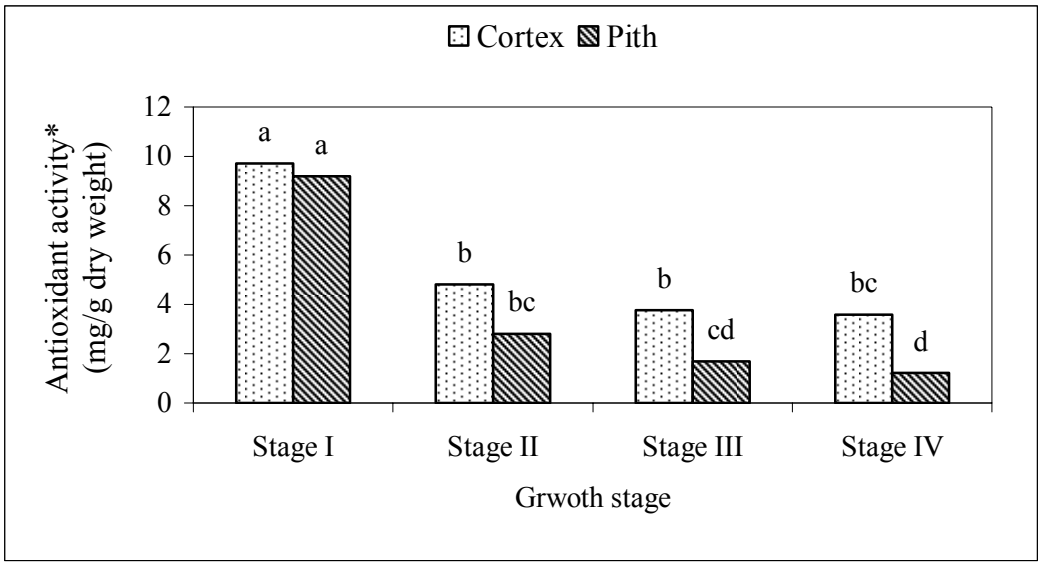

Figure 3.3. Antioxidant activity in sweetpotato root tissues at different growth stages. Means with different letters are significantly different at P<0.05 (Tukey's test).*Data expressed as mg of Trolox equivalents per g of dry weight.

although no significant difference was found at stage I and II. The pith tissue of fully developed roots at stage IV had the lowest antioxidant activity (1.2 mg/g dry weight).

Wang and Lin (2000) reported the small green stage in a developing strawberry fruit had the highest antioxidant activity.

The antioxidant activity of sweetpotato leaves was significantly higher than the root tissues. The highest antioxidant activity (99.6 mg/g dry weight) found in young leaves was almost 10 times higher than the highest root tissue antioxidant activity (9.7 mg/g dry weight in cortex tissue of roots at stage I). The antioxidant activity of sweetpotato leaves was also significantly affected by leaf age. Antioxidant activity of immature young leaves (99.6 mg/g dry weight) was significantly higher than mature (31.1 mg/g dry weight) and old leaves (21.0 mg/g dry weight) (Figure 3.2). Concomitant with the lowest total phenolic content, the petiole also had the lowest antioxidant activity (9.6 mg/g dry weight). Wang and Lin (2000) also reported the young leaves from berry crops had higher antioxidant activity than older leaves.

The correlation coefficients (r^2) between antioxidant activity and total phenolic content of root and leaf tissue at different growth stages were 0.979 and 0.998, respectively. Strong correlation coefficients indicate that antioxidant activity of sweetpotato root and leaf tissue increases with increased total phenolic content. Previously, Islam et al. (2003a) reported a positive correlation between antioxidant activity and total phenolic content in sweetpotato leaves.

Individual Phenolic Acids

Typical HPLC chromatograms from sweetpotato roots (stage I) and old leaves are shown in Figure 3.4. Five different individual phenolic acids (ChlA; CafA; 4,5-diCQA; 3,5-diCQA; 3,4-diCQA) were identified in both sweetpotato root and leaf tissues. As indicated in previous reports, the unknown peaks were assumed to be isomers of

chlorogenic acid and 3,4,5-tricaffeoylquinic acid but need further confirmation (Islam et al. 2002; Walter et al. 1979). Results for individual phenolic acids content in sweetpotato roots and leaves at different growth stages are summarized in Tables 3.2 and 3.3. Chlorogenic acid was the most abundant phenolic acid in root tissue, followed by 3,5-diCQA. The trend of change in individual phenolic acids paralleled the total phenolic content. Islam et al. (2003b) reported a positive correlation between caffeoylquinic acid

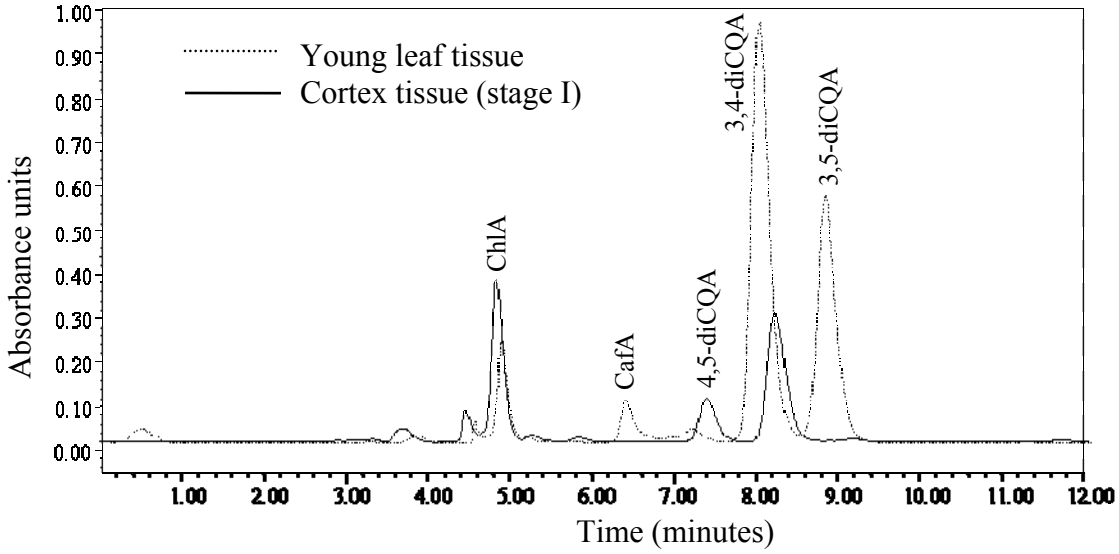

Figure 3.4. Comparison of HPLC chromatograms for individual phenolic acids isolated from young leaf tissue and cortex tissue (stage I). ChlA: chlorogenic acid; CafA: caffeic acid; 4,5-diCQA: 4,5-dicaffeoylquinic acid; 3,5-diCQA: 3,5-dicaffeoylquinic acid; 3,4-diCQA: 3,4-dicaffeoylquinic acid.

derivatives and total phenolic content of sweetpotato leaves. In general, roots in the initial stages of development had higher amounts of individual phenolic acids, which decreased with increasing root size. The amounts of all identified individual phenolic acids, except 4,5-diCQA and 3,4-diCQA, were significantly higher in roots at stage I compared to stage IV. Both cortex and pith tissue at stage I had significantly higher amounts of caffeic acid than the other stages of development. At all stages of development, cortex tissue had

higher amounts of individual phenolic acids (although not always significant) than the pith tissue. A recent report indicated chlorogenic acid in young pear fruit was significantly higher than in fully mature ripe fruit (Cui et al., 2005). Buta and Spaudling (1997) reported the decline in chlorogenic acid content during growth and development of tomato fruit paralleled the decline in indole-3-acetic acid level.

Table 3.2. Individual phenolic acid content in sweetpotato root tissues at different growth stages.

Growth stage	Tissue Type	ChlA	CafA	4,5-diCQA	3,5-diCQA	3,4-diCQA
		(mg/100 g dry weight)				
Stage I	Cortex	164.2^{a}	2.7^{a}	72.1^{a}	157.6^{a}	10.4^{ab}
	Pith	141.9^{ab}	2.3^{a}	69.3^{ab}	136.0^{a}	27.7^{a}
Stage II	Cortex	98.9^{bc}	0.5^{b}	27.9^{bc}	62.9^{b}	6.0^{b}
	Pith	54.6^{cd}	0.4^{b}	14.6^{bc}	28.6^{bcd}	4.9^{ab}
Stage III	Cortex	87.8^{c}	0.6^{b}	17.3^{c}	59.7^{bc}	6.8^{b}
	Pith	38.4^{d}	0.4^{b}	7.6^{bc}	20.4^{cd}	3.4^{ab}
Stage IV	Cortex	89.4^{c}	0.6^{b}	21.9^{bc}	53.4^{bc}	6.7^{b}
	Pith	34.4^{d}	0.3^{b}	5.8^{bc}	14.3^{d}	2.3^{b}

ChlA:chlorogenic acid; CafA:caffeic acid; 4,5-diCQA:4,5-dicaffeoylquinic acid; 3,5-diCQA:3,5-dicaffeoylquinic acid; 3,4-dicaffeoylquinic acid. Mean values (n=5) with same letters in the same column are not significantly different at P<0.05 (Tukey's test).

Table 3.3. Individual phenolic acid content in sweetpotato leaf tissues at different growth stages.

Growth stage	ChlA	CafA	4,5-diCQA	3,5-diCQA	3,4-diCQA
	(mg/100 g dry weight)				
Young	632.0^{a}	120.2^{a}	140.9b^{c}	2998.7^{a}	2890.8^{a}
Mature	444.9^{b}	16.3^{b}	319.3^{a}	354.8^{b}	342.3^{b}
Old	300.1^{c}	8.4^{b}	209.3^{b}	112.6^{c}	139.6^{c}
Petiole	114.8^{d}	2.7^{b}	103.6^{c}	114.0^{c}	54.0^{c}

ChlA:chlorogenic acid; CafA:caffeic acid; 4,5-diCQA:4,5-dicaffeoylquinic acid; 3,5-diCQA:3,5-dicaffeoylquinic acid; 3,4-dicaffeoylquinic acid. Mean values (n=5) with same letters in the same column are not significantly different at P<0.05 (Tukey's test).

Individual phenolic acid content in sweetpotato leaves was significantly higher than in root tissue, which was consistent with a previous report (Islam et al., 2002). Our results also indicated the leaf phenolic acid profile varied according to the leaf age. In general, young leaves had higher amounts of individual phenolic acids than mature and

old leaves, except 4,5-diCQA, which was significantly lower in young leaves than mature and old leaves. Petiole tissue had lower amounts of phenolic acids than leaf blade. The most abundant phenolic acid in young leaves was 3,5-diCQA, whereas ChlA was the major phenolic acid in mature and old leaves. Previously, the 3,5-diCQA had been reported as the most abundant phenolic acid in sweetpotato leaves (Islam et al., 2002). This inconsistency in results may be due to genotype differences or age of leaves used for analysis. Considering the high antioxidant properties of caffeoylquinic acids, consumption of young sweetpotato leaves may be more beneficial than mature and old leaves.

CONCLUSIONS

This study may have useful implications for the functional food industry. Results indicated that immature root and leaf tissues may be potentially used as concentrated sources of nutarceutical compounds. Consumption of functional foods originating from immature sweetpotato root and leaf tissues high in phenolic content may have significant positive health benefits. Also, these tissues may be potentially concentrated sources of caffeoylquinic acids, useful to the pharmaceutical industry. Further research to elucidate the mechanisms involved in higher phenolic synthesis in immature tissues would be useful to enhance the nutraceutical value of sweetpotatoes.

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CHAPTER 4. PHENOLIC ACID COMPOSITION AND ANTIOXIDANT ACTIVITY OF PROCESSED SWEETPOTATOES

INTRODUCTION

Sweetpotato [*Ipomoea batatas* (L.) Lam.] is a major food crop in developing countries with a total world production of approximately 130 million tons (FAOSTAT 2005). It ranks as the world's seventh most important food crop. Phenolic compounds are important secondary metabolites present in sweetpotatoes. Their antioxidant properties have been implicated in suppressing various health related disorders, including melanogenesis (Shimozono et al., 1996), hepatoma invasion (Yagasaki et al., 2000), and human immunodeficiency virus (HIV) replication (Mahmood et al., 1993; Zhu et al., 1999). The antioxidant potential of phenolic compounds in sweetpotato root tissue has been evaluated and reported to be higher than in many vegetables (Yamakawa and Yoshimoto, 2002; Cevallos-Casalas and Cisneros-Zevallos, 2002).

Sweetpotato phenolics were first isolated by Rudkin and Nelson (1947), who identified chlorogenic acid as the major phenolic acid in the root tissue. Chlorogenic acid, caffeic acid, and isomers of dicaffeoylquinic acid were identified as the main phenolic acids in several different sweetpotato cultivars (Walter and Schadel, 1981; Son et al., 1991). These individual acids have been reported to possess a strong antimutagenic and antioxidative activity against free radicals (Yamada and Tomita, 1996; Yoshimoto et al., 1999). Recently, the antioxidant activity of several sweetpotato cultivars was found to be highly correlated with the phenolic acids such as chlorogenic acid and isochlorogenic acid (Oki et al., 2002; Huang et al., 2004).

Although the sweetpotato may be considered as a rich source of these antioxidant compounds, distribution of phenolics in the root varies depending on tissue location and

type such as periderm (outer skin), cambium layer, and inner pith tissue. Antioxidant activity and total phenolic compounds in sweetpotato skin tissue were found to be higher than in internal root tissue (Walter and Schadel, 1981; Cevallos-Casalas and Cisneros-Zevallos, 2002; Truong et al., 2004). Harrison et al. (2003) reported much lower levels of caffeic acid in cortex tissue as compared to periderm tissue. The high phenolic content in skin tissue may have implications in utilization of waste peelings from processed sweetpotatoes.

In addition to tissue level differences, processing conditions may affect the phenolic content in the edible product. Microwaving, conventional oven baking, and boiling are the most widely used methods of sweetpotato processing at home and in food-service establishments. Phenolic compounds were found to be destroyed during heating (Philpott et al., 2003). The loss of chlorogenic acid during heating of potato has also been reported (Dao and Friedman, 1992). Caffeic acid derivatives in potato were reported to be better retained by steam cooking compared to other cooking methods (Tudela et al., 2002). Total phenolic content and individual phenolic acid composition of raw sweetpotato roots has been previously reported. However, in most parts of the world, sweetpotato is consumed after some type of cooking method. Little information is available on the changes in phenolic composition and antioxidant activity of sweetpotatoes during processing. The objective of this study was to determine the effect of processing method on phenolic content and antioxidant activity in sweetpotato tissue.

MATERIALS AND METHODS

Reagents

Chlorogenic and caffeic acid standards, Folin-Denis reagent, and DPPH (1, 1-diphenyl-2-picrylhydazyl) reagent were purchased from Sigma-Aldrich (St. Louis, MO). Standards of three isomers (4,5-dicaffeoylquinic acid; 3,5-dicaffeoylquinic acid; and 3,4-dicaffeoylquinic acid) of isochlorogenic acid were kindly provided by Dr. Makoto Yoshimoto (Department of Upland Farming, Kyushu National Agricultural Experiment Station, Miyakonojo, Miyazaki, Japan).

Plant Material

'Beauregard' sweetpotato [*Ipomoea batatas* (L.) Lam.] roots were harvested in early October, 2004 from the LSU AgCenter Sweetpotato Research Station in Chase, La. They were immediately cured after harvest at 30 °C and 90% relative humidity for 7 days and then stored at 15 °C until analyzed. Randomly selected sound roots, approximately 300 g in weight, were obtained from storage, washed, and allowed to dry at ambient temperature (~21 °C).

Tissue Preparation and Extraction

Individual sweetpotato roots were cut in half longitudinally, followed by transversely into four sections about 3-4 cm thick. One section was used for raw tissue analysis and the remaining three sections were subjected to three heat processing methods: (a) microwaving at 1500 Watts for 10 minutes; (b) baking in a conventional oven at 190 °C for 45 minutes; (c) boiling at 100 °C in water for 25 minutes. Each section was further divided into 3 tissue types: outer skin tissue, cortex, and central pith tissue. Exactly 1 g of tissue was obtained from each tissue type by excision with a scalpel. The

tissue was put into a 15 ml Falcon tube and homogenized in 80% methanol using a VirTishear homogenizer (Virtis Co., Gardiner, NY). Approximately 8 ml of 80% methanol was added and the capped tubes were heated at 80 °C for 10 minutes. After vigorously shaking the heated samples by hand, the tubes were cooled and centrifuged at 4,500 g for 15 minutes. The supernatant was decanted and volumes were adjusted to 10 ml prior to analyses of total phenolic content and individual phenolic acids.

Total Phenolics

Total phenolic content was determined by modification of the Folin-Denis method (Swain and Hillis, 1959). Exactly 0.5 ml of supernatant was placed in a 25 ml test tube and mixed with 8 ml of Megapure water (Barnstead MP-12A, Haverhill, MA) followed by the addition of 0.5 ml of Folin-Denis reagent. After 3 minutes, 1 ml of 1 N Na_2CO_3 was added and the solution was allowed to stand for 2 hours at ambient temperature (~21 °C). Absorbance of the resulting blue complex was measured at 750 nm using a Lambda 35 UV/Vis spectrophotometer (PerkinElmer Instruments, Norwalk, CT). A standard curve of chlorogenic acid (50-300 µg/ml concentration) was plotted. The total phenolic content was expressed as mg chlorogenic acid equivalent/g dry weight.

Antioxidant Activity

The antioxidant activity was measured according to Brand-Williams et al. (1995), with slight modifications. DPPH (1, 1-diphenyl-2-picrylhydazyl) was used as the source of free radicals. The absorbance of free radicals at 520 nm disappears upon their reduction by an antioxidant. In this study, Trolox (6-hydroxy-2, 5, 7, 8-tetramethyl-chroman-2-carboxylic acid) was used as the standard antioxidant compound. An aliquot (300 µl) of sweetpotato extract was placed in a 1.5 ml amber colored centrifuge tube.

Then 600 µl of 0.1 M MES [2-(N-morpholine) ethanesulfonic acid, pH 6.0] and 300 µl of 0.4 mM of DPPH solution were added to the centrifuge tube. The resultant mixture was shaken and held in the dark for 2 minutes. The decrease in DPPH absorbance was measured at 520 nm using a Lambda 35 UV/Vis spectrophotometer. The sweetpotato extract was replaced with 80% methanol in the control samples. For the blank, 80% methanol without DPPH was used. The antioxidant activity was calculated from a standard curve made with known concentrations of Trolox and expressed in terms of mg Trolox equivalent/g dry weight.

Individual Phenolic Acids

Separation of individual phenolic acids was accomplished using reversed-phase HPLC with a Gemini C18, 5 µm, 4.6 × 250 mm (Phenomenex Inc., Torrance, CA) column. An aliquot of the supernatant was filtered through a 0.45 µm Nylaflo membrane filter (Pall Corp., East Hills, NY). Twenty µl of filtrate was injected onto the column using a Waters 717 autosampler connected to a Waters 600E pump (Waters Corp., Milford, MA). Phenolic acids were eluted using a mobile phase consisting 1% (v/v) formic acid in aqueous solution: acetonitrile: 2-propanol (70:22:8), pH 2.5, with an isocratic flow rate of 0.75 ml/min. Peaks detected were identified and quantified by comparing the retention time and peak area to that of known standards. Quantification was based on absorbance at 320 nm using a Waters 2487 dual wavelength UV absorbance detector.

Statistical Analysis

A completely randomized design was used with five replications per treatment. Each root was considered a replication. Data was analyzed by SAS procedure MIXED

(SAS Institute Inc., Cary, NC). A Pearson correlation was used to determine the relationship between total phenolic content and antioxidant activity of sweetpotato root tissues.

RESULTS AND DISCUSSION

Total Phenolics

Total phenolic content was highest in the skin tissue (raw or processed) compared to the other tissue types. The raw sweetpotato skin tissue had the highest total phenolic content (17.7 mg/g dry weight) (Table 4.1). This was consistent with a previous report with 'Jewel' sweetpotatoes (Walter and Schadel, 1981). However, our results indicated

Table 4.1. Total phenolics in raw and processed sweetpotato root tissues.

	Total phenolics*		
Treatment	Skin	Cortex	Pith
Raw	17.7[a]	3.7[c]	2.1[c]
Microwaved	10.3[b]	3.0[c]	1.8[c]
Boiled	11.0[b]	3.8[c]	1.6[c]
Baked	7.9[b]	4.2[c]	2.1[c]

Mean values (n=5) with same letters are not significantly different at P<0.05. *Data expressed as mg of chlorogenic acid equivalents per g of dry weight.

that thermal processing of the sweetpotato skin resulted in a significant loss of total phenolic content. The lowest total phenolic content was observed in boiled pith tissue (1.6 mg/g dry weight), although differences were not significant. Total phenolic content in sweetpotato skin tissue was reduced by 42% after microwaving, 55% after conventional oven baking, and 37% after boiling. Differences in total phenolic content among the three processing methods were not significant. No significant loss of total phenolic content was observed due to heat processing of cortex and pith tissue. Among various potato processing methods, baking resulted in the greatest loss of chlorogenic acid (Dao and Friedman, 1992). It is not known whether heat breaks down the phenolics

or whether they form larger macromolecules with other compounds present in the sweetpotato tissue. Heating may disrupt the intra-cellular separation of the phenolic acids and oxidative enzymes (polyphenoloxidases), resulting in degradation of the phenolic acids.

Antioxidant Activity

The results presented in Table 4.2 indicated the antioxidant activity was highest in raw skin tissue (22.9 mg/g dry weight). The conventional oven baked skin tissue had a significantly lower antioxidant potential compared to microwaved or boiled tissue. This may be due to the lower total phenolic content in conventional oven baked skin tissue. No significant difference was detected in the antioxidant potential of microwaved and boiled skin tissue. None of the processing treatments resulted in a significant loss of antioxidant activity in cortex and pith tissue. Boiled pith tissue contained the lowest antioxidant activity (1.7 mg/g dry weight), although no significant differences were observed.

Table 4.2. Antioxidant activity in raw and processed sweetpotato root tissues.

	Antioxidant activity*		
Treatment	Skin	Cortex	Pith
Raw	22.9a	5.5c	2.3c
Microwaved	13.7b	5.7c	1.9c
Boiled	14.1b	4.3c	1.7c
Baked	6.3c	3.8c	1.9c

Mean values (n=5) with same letters are not significantly different at $P<0.05$. *Data expressed as mg of Trolox equivalents per g of dry weight.

Our results are consistent with the findings of Philpott et al. (2003) who reported baking and boiling of sweetpotato skin tissue resulted in a higher reduction of antioxidant activity than in raw skin tissue. This study indicated the pattern of change in antioxidant activity of sweetpotato root tissue paralleled that of total phenolic content. A strong

correlation (r^2=0.98) existed between total phenolic content and antioxidant activity (Figure 4.1).

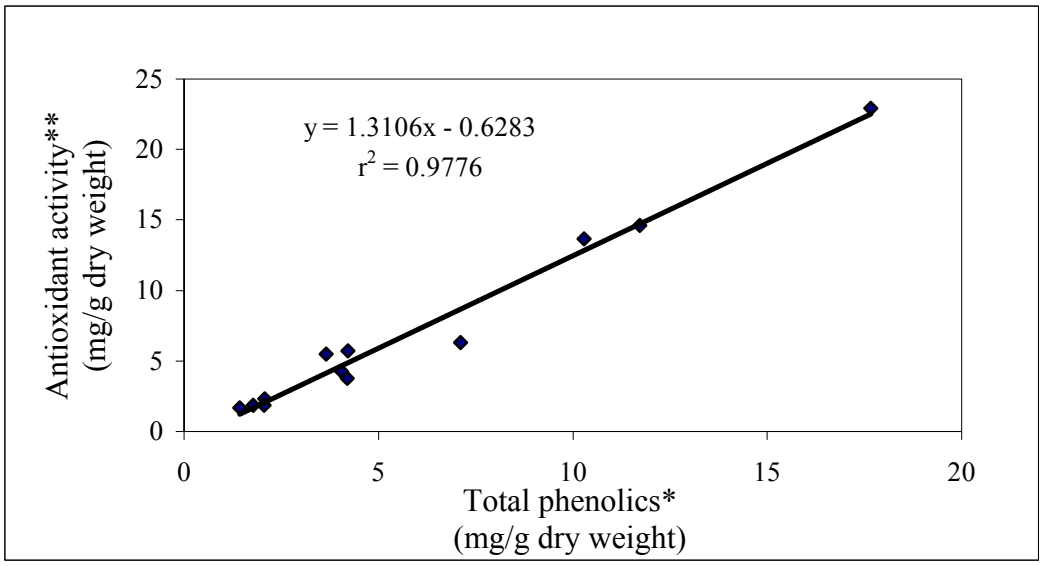

Figure 4.1. Correlation between antioxidant activity and total phenolics in sweetpotato root tissues. *Data expressed as mg of chlorogenic acid equivalents per g of dry weight. **Data expressed as mg of Trolox equivalents per g of dry weight.

Individual Phenolic Acids

Chlorogenic acid, caffeic acid, and three isomers of the dicaffeoylquinic acids (3, 4-dicaffeoylquinic; 3,5-dicaffeoylquinic; and 4,5-dicaffeoylquinic acid) were identified as the principal phenolic acids in sweetpotato root tissue. The results for individual phenolic acid content in processed sweetpotato tissues presented in Tables 4.3-4.5. The outer skin tissue (raw or processed) had the highest amount of individual phenolic acids followed by the cortex tissue. The pith tissue had the lowest content of individual phenolic acids.

Chlorogenic acid was the most abundant individual phenolic acid present in sweetpotato root tissues, consistent with the previous finding of Walter et al. (1979). We found that thermal processing resulted in significant loss of phenolic acids present in

sweetpotato skin tissue. Raw skin tissue was found to contain a significantly higher amount (4.64 mg/g dry weight) of chlorogenic acid compared to microwaved (2.58 mg/g dry weight), baked (1.34 mg/g dry weight) and boiled (1.60 mg/g dry weight) skin tissue

Table 4.3. Individual phenolic acid content in raw and processed sweetpotato skin tissue.

Treatment	ChlA	CafA	4,5-diCQA	3,5-diCQA	3,4-diCQA
	mg/g dry weight				
Raw	4.64[a]	0.91[a]	0.78[a]	1.78[a]	1.44[a]
Microwaved	2.58[b]	0.16[b]	0.66[a]	1.08[b]	1.02[a]
Boiled	1.60[c]	0.07[b]	0.68[a]	0.59[b]	1.48[a]
Baked	1.34[c]	0.08[b]	0.41[b]	0.41[b]	0.86[a]

Mean values (n=5) with same letters in a column are not significantly different at P<0.05.

Table 4.4. Individual phenolic acid content in raw and processed sweetpotato cortex tissue.

Treatment	ChlA	CafA	4,5-diCQA	3,5-diCQA	3,4-diCQA
	mg/g dry weight				
Raw	0.95[a]	0.02[a]	0.16[a]	0.57[a]	0.60[a]
Microwaved	1.67[a]	0.07[a]	0.50[a]	0.53[a]	0.83[a]
Boiled	0.71[a]	0.02[a]	0.24[a]	0.19[a]	0.50[a]
Baked	0.76[a]	0.03[a]	0.23[a]	0.18[a]	0.47[a]

Mean values (n=5) with same letters in a column are not significantly different at P<0.05.

Table 4.5. Individual phenolic acid content in raw and processed sweetpotato pith tissue.

Treatment	ChlA	CafA	4,5-diCQA	3,5-diCQA	3,4-diCQA
	mg/g dry weight				
Raw	0.46[a]	0.01[a]	0.04[a]	0.16[a]	0.21[a]
Microwaved	0.34[a]	0.01[a]	0.06[a]	0.06[a]	0.13[a]
Boiled	0.20[a]	0.01[a]	0.04[a]	0.03[a]	0.09[a]
Baked	0.26[a]	0.01[a]	0.04[a]	0.03[a]	0.09[a]

Mean values (n=5) with same letters in a column are not significantly different at P<0.05.

(Table 4.3). None of the heat processing methods had a significant effect on chlorogenic acid content in the cortex or pith tissue (Tables 4.4 and 4.5). An unidentified peak eluted immediately before chlorogenic acid and was assumed to be an isomer of chlorogenic acid. The size of this unknown peak increased significantly after heat processing. A decrease in chlorogenic acid and concomitant increase in the unknown isomer suggest

that heating might have caused conversion of some chlorogenic acid to this unknown isomer.

Among all the individual phenolic acids identified, caffeic acid was present in the lowest concentration, except in the skin tissue, where concentration of caffeic acid was higher than 4,5-dicaffeoylquinic acid. The caffeic acid content of raw skin tissue (0.91 mg/g dry weight) was significantly higher than in the cortex (0.02 mg/g dry weight) and pith tissue (0.01 mg/g dry weight). This is consistent with the results of Harrison and others (2003), who reported the periderm and cortex tissue of 'Beauregard' sweetpotato had an average caffeic acid content of 0.66 mg and 0.01 mg/g dry weight, respectively. The higher level of caffeic acid in the skin tissue was thought to be a chemical defense mechanism against fungal pathogens. Our results indicated a significant loss of caffeic acid due to heat processing of skin tissue.

The average concentrations of the three isomers of dicaffeoylquinic acid in raw sweetpotato skin tissue were: 3,5-diCQA (1.78 mg/g); 3,4-diCQA (1.44 mg/g); and 4,5-diCQA (0.78 mg/g). Skin tissue had significantly higher amounts of dicaffeolquinic acids than the other tissue types. No significant difference was observed among cortex and pith tissue. Heat processing tended to decrease the content of dicaffeoylquinic acids. A significant loss of 3,5-dicaffeoylquinic acid occurred in boiled or baked skin tissue. Individual phenolic acids in cortex and pith tissues were less affected by the type of processing.

CONCLUSIONS

Heat processing decreased the phenolic acid content and antioxidant activity of sweetpotato root tissues. Conventional baking resulted in the greatest loss of total

phenolic content and antioxidant activity in skin tissue, although differences were not signifcicant for total phenolic content. Sweetpotato skin tissue, processed or raw, had the highest phenolic acid content and antioxidant potential among three tissue types. Sweetpotato skin may be a potentially good source of nutraceutical compounds important in reducing the incidence of certain human health-related illnesses.

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CHAPTER 5. EFFECT OF LOW TEMPERATURE STORAGE ON PHENOLIC COMPOSITION AND ANTIOXIDANT ACTIVITY OF SWEETPOTATOES

INTRODUCTION

Sweetpotato is indigenous to tropical regions and suffers from chilling injury when subjected to low temperature storage. Chilling injury may occur during on-farm storage, during long distance transport, in wholesale and retail storage facilities, in supermarket display racks, and in consumer refrigerators. Long-term exposure to low temperature results in internal tissue darkening of sweetpotato roots, which has been attributed to an increased content of phenolic compounds (Lieberman et al., 1958; Lieberman et al., 1959; Porter et al., 1976). A significant increase in chlorogenic acid content and polyphenols was reported in chilled 'Porto Rico' sweetpotato roots (Lieberman et al., 1958). However, Minamikawa et al. (1961) found no significant increase in chlorogenic acid content or polyphenols in 'Okinawa No. 100' sweetpotato roots stored at 0 °C. These apparent contradictions indicate chilling injury and phenolic content in sweetpotatoes is influenced by cultivar and other pre- and/or postharvest conditions. Cantwell et al. (2002) reported an accelerated buildup of phenolics when jicama roots were transferred to 20 °C after 2 weeks of storage at 10 °C. They suggested that a transfer to warmer temperature intensifies the changes induced by low temperature storage. The extent of chilling injury symptoms is influenced by curing, duration of exposure to low temperature, and the specific exposure temperature. Picha (1987) reported external chilling injury symptoms and internal tissue darkening was greater in non-cured than cured sweetpotato roots.

The change in phenolic compound metabolism in response to low temperature depends on the tissue sensitivity. Yamaki and Uritani (1972) reported vacuolar membrane degradation in some regions of the sweetpotato root tissue. This may have resulted in a greater influx of phenolic compounds into the cytosol from their vacuolar storage location. Phenolic compounds build up gradually as a result of chilling and are oxidized in presence of air to form dark pigments (Lieberman et al., 1958). Recently, phenolic compounds have attracted attention due to their antioxidant properties and ability to protect the human body from oxidative stress (Kaul and Khanduja, 1998). Thus an increase in phenolic compounds due to low temperature exposure may enhance the nutraceutical value of sweetpotato roots. However, an extended period of low temperature exposure may result in chilling injury, which decreases the market value of sweetpotato roots. No information exists on the use of limited low temperature exposure times for enhancement of nutraceutical value in sweetpotato roots. The objective of this study was to determine the effect of low temperature storage on phenolic acid composition in different tissues of cured and non-cured sweetpotato roots.

MATERIALS AND METHODS

Reagents

Chlorogenic acid and caffeic acid standards, Folin-Denis reagent and DPPH (1, 1-diphenyl-2-picrylhydazyl) reagent were purchased from Sigma-Aldrich (St. Louis, MO). Standards of three isomers of dicaffeoylquinic acid (4,5-diCQA, 3,5-diCQA, 3,4-diCQA) were kindly provided by Dr. Makoto Yoshimoto (Department of Upland Farming, Kyushu National Agricultural Experiment Station, Miyakonojo, Miyazaki, Japan).

Plant Material

'Beauregard' sweetpotatoes were grown at the LSU AgCenter Sweetpotato Research Station in Chase, LA in 2004. The roots were harvested during the first week of October and divided into two lots. One lot was cured at 30 °C and 90% relative humidity for 7 days and then stored at 5 °C and 15 °C. The second lot was stored at 5 °C without curing. The roots were sampled at harvest, after curing, and at specified intervals during a 4-week exposure to low temperature.

Tissue Preparation and Extraction

In an initial experiment, cured and non-cured sweetpotato roots were analyzed for total phenolic content at weekly intervals during a 4-week storage period at 5 °C. Randomly selected roots were washed and allowed to dry at ambient temperature before tissue preparation. Roots were blended using a Cuisinart model DLC-2AR food processor (Cuisinart Inc., Windsor, NJ). Blended tissue was passed through a 1 mm sieve and exactly 1 g of sieved tissue was placed in a 15 ml Falcon centrifuge tube. Approximately 8 ml of 80% methanol was added to the centrifuge tube. The tubes were capped and immersed in a water bath at 80 °C for 10 minutes. After vigorously shaking the heated samples manually for 30 seconds, the tubes were cooled and centrifuged at 4,500 g for 15 minutes. The final volume of clear supernatant was made to 10 ml with 80% methanol and analyzed for total phenolic content.

A second experiment was initiated to study the effect of chilling temperature on total phenolic content and individual phenolic acid content in different root tissues in non-cured roots. Chilled roots were analyzed after 2 and 4 weeks at 5 °C. At each sampling, some of the chilled roots were held at 22 °C for an additional 3 days. The four

tissue types analyzed at each sampling were: skin (periderm), cortex, cambium, and pith. Exactly 1 g of tissue, excised with a scalpel, was obtained from each tissue type from the same root. The individual tissues were put in 15 ml Falcon tubes and homogenized in 80% methanol using a VirTishear homogenizer (Virtis Co., Gardiner, NY). Approximately 8 ml of 80% methanol was added to the tubes. The capped tubes were immersed in a water bath at 80 °C for 10 minutes and manually shaken for 30 seconds. The supernatant was collected after centrifugation and analyzed for total phenolic content and individual phenolic acids.

Total Phenolics

Total phenolic content was determined by modification of the Folin-Denis method (Swain and Hillis, 1959). Exactly 0.5 ml of supernatant was placed in a 25 ml test tube and mixed with 8 ml of Megapure water (Barnstead MP-12A, Haverhill, MA) followed by the addition of 0.5 ml of Folin-Denis reagent. After 3 minutes, 1 ml of 1 N Na_2CO_3 was added and the solution was allowed to stand for 2 hr at 22 °C. Absorbance of the resulting blue color complex was measured at 750 nm using a Lambda 35 UV/Vis spectrophotometer (PerkinElmer Inst., Norwalk, CT). The total phenolic content was expressed as mg chlorogenic acid equivalents/100 g fresh weight.

Antioxidant Activity

The antioxidant activity was measured according to the method developed by Brand-Williams et al. (1995), with slight modifications. DPPH (1, 1-diphenyl-2-picrylhydazyl) was used as the source of free radicals. The absorbance of free radicals at 520 nm disappears upon their reduction by an antioxidant. In this study, Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) was used as the standard

antioxidant compound. An aliquot (300 µl) with a suitable dilution of sweetpotato extract was placed in a 1.5 ml amber colored centrifuge tube. Then 600 µl of 0.1 M 2-(N-morpholine) ethanesulfonic acid (MES) (pH 6.0) and 300 µl of 0.4 mM of DPPH solution were added to the centrifuge tube. The resultant mixture was shaken and held in the dark for 2 minutes. The decrease in DPPH absorbance was measured at 520 nm using a Lambda 35 UV/Vis spectrophotometer (PerkinElmer Inst., Norwalk, CT). The sweetpotato extract was replaced with 80% methanol in the control samples. For the blank, 80% methanol without DPPH was used. The antioxidant activity was calculated from a standard curve made with known concentrations of Trolox and expressed in terms of mg Trolox equivalents/100 g fresh weight.

Individual Phenolic Acids

Separation, analysis and quantification of individual phenolic acids were accomplished using reversed-phase HPLC with a Gemini C18, 5 µm, 250 × 4.6 mm (Phenomenex, Torrance, CA) column. An aliquot of the supernatant was filtered through a 0.45 µm Nylaflo membrane filter (Pall Corp., East Hills, NY). A sample volume of 20 µl was injected onto the column using a Waters 717 autosampler connected to a Waters 600E pump (Waters Corp., Milford, MA). Phenolic acids were eluted using a mobile phase consisting of 1% (v/v) formic acid in aqueous solution: acetonitrile: 2-propanol (70:22:8) at pH 2.5 with an isocratic flow rate of 0.75 ml/min. Peaks detected were identified and quantified by comparing the retention time and peak area to that of known standards. Quantification was based on absorbance at 320 nm using a Waters 2487 dual wavelength UV absorbance detector.

Statistical Analysis

A completely randomized design with five replications per treatment was used. Each root was considered as one replication. Data was analyzed by MIXED procedure methods (SAS Institute Inc., Cary, NC). Means were separated using Tukey's test ($P<0.05$). A Pearson correlation was used to determine the relationship between total phenolic content and antioxidant activity of sweetpotato root tissues.

RESULTS AND DISCUSSION

Total Phenolics

A significant increase in total phenolic content of non-cured and cured roots was observed after 2 and 3 weeks of low temperature exposure, respectively. However, long term storage (12 months) of cured sweetpotatoes at 15 °C resulted in no significant change in total phenolic content (data not shown). Our results showed that total phenolic content increased at a higher rate in non-cured roots than in cured roots during low temperature storage (Figure 5.1). After each week of low temperature exposure, non-cured roots had a higher total phenolic content than cured roots. However, a significant difference was observed only after 4 weeks exposure at 5 °C. Total phenolic content after 4 weeks of low temperature exposure increased from 55.3 mg/100g fresh tissue (at harvest) to 79.4 mg/100 g fresh tissue in cured roots compared to 125.8 mg /100 g fresh tissue in non-cured roots. Total phenolic content in non-cured roots exposed to 4 weeks of chilling was significantly higher than all other treatments. Internal tissue browning in non-cured roots was apparent after 3 weeks of 5 °C exposure. Non-cured tissue was noticeably darker than cured tissue. Our results are consistent with Picha (1987), who reported darkening of the cambium and vascular bundles in non-cured 'Whitestar' and

'Rojo Blanco' sweetpotato roots exposed to 7 °C for 3 weeks. Lieberman et al. (1959) and Porter et al. (1976) also reported internal tissue browning of sweetpotato roots

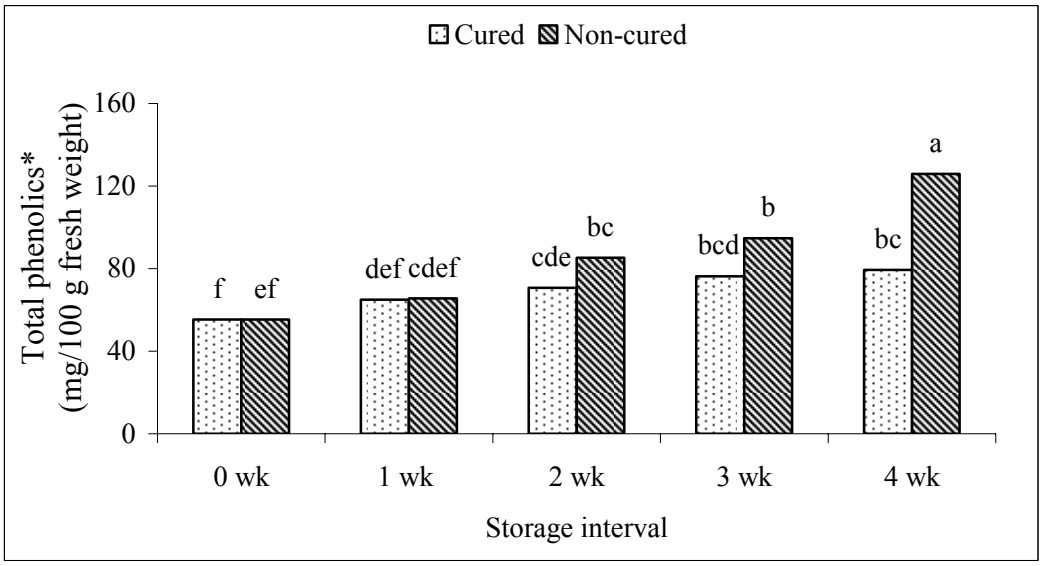

Figure 5.1. Total phenolics in cured and non-cured sweetpotato roots at different intervals of storage at 5 °C. Means with different letters are significantly different at $P<0.05$ (Tukey's test). *Data expressed as mg of chlorogenic acid equivalents per 100 g of fresh weight.

exposed to chilling temperatures. The darkening of chilled sweetpotato tissue was attributed to increased content of phenolic compounds.

A second experiment was undertaken to quantify the total phenolic content in different sweetpotato root tissues. Total phenolic content of freshly harvested roots was highest in skin tissue (279.7 mg/100 g fresh weight) and lowest in pith tissue (44.9 mg/100 g fresh weight) (Table 5.1). Previously, Walter and Schadel (1981) had reported the outer skin of sweetpotato contained higher amounts of phenolics compared to inner pith tissue. The higher amount of phenolics in skin tissue of sweetpotato was attributed to the natural chemical defense mechanism against diseases and insect pests (Harrison et al., 2003). Our results indicated the low temperature exposure resulted in an increase in total phenolic content in all tissue types. Transfer to ambient temperature after 2 and 4 weeks

of low temperature storage resulted in increased total phenolic content in all tissue types. Depending on tissue type, the total phenolic content in freshly harvested non-cured sweetpotato roots increased two- to three-fold when roots were exposed to 3 days of warmer temperature after 4 weeks at 5 °C (Table 5.1). However, irrespective of the low temperature storage duration, skin tissue always had a significantly higher total phenolic content than other tissues. No significant difference in phenolic content was observed between cortex and cambium tissue, whereas, pith had a significantly lower total phenolic content. A significant increase in total phenolic content of cortex, cambium, and pith tissue was observed after 3 days of 22 °C exposure following 4 weeks at 5 °C. Cantwell et al. (2002) also observed similar results is jicama roots, where they reported an accelerated buildup of phenolics when roots stored at 10 °C for 2 weeks were transferred to 22 °C. The increase in total phenolic concentration paralleled the increased phenylalanine ammonia-lyase activity (PAL), an important enzyme in the phenylpropanoid pathway involved in phenolic synthesis.

Table 5.1. Total phenolics in different tissues of non-cured sweetpotato roots at different intervals of storage at 5 °C, including transfer to ambient temperature (22 °C) for an additional 3 days (d).

Storage interval	Total phenolics*			
	Skin	Cortex	Cambium	Pith
0 wk	279.7^{c}	117.7^{b}	139.4^{b}	44.9^{b}
2 wk	356.4^{c}	151.5^{b}	168.0^{b}	49.0^{b}
2 wk + 3 d	407.7^{bc}	153.6^{b}	157.6^{b}	47.4^{b}
4 wk	507.9^{ab}	195.6^{ab}	234.1^{ab}	64.4^{ab}
4 wk + 3 d	755.1^{a}	280.0^{a}	466.9^{a}	82.9^{a}

Means with same letters in a column are not significantly different at $P<0.05$ (Tukey's test). *Data expressed as mg of chlorogenic acid equivalents per 100 g of fresh weight.

Stress induced phenolic compounds may be the result of increased transcription of genes encoding the corresponding biosynthetic enzymes (Dixon and Paiva, 1995). They

suggested the specific heterotetrameric forms of PAL, a tetrametric enzyme encoded by multiple genes, depends on the type of stress.

Antioxidant Activity

The increase in antioxidant activity of sweetpotato roots with increasing lengths of exposure to 5 °C was consistent with the trend for total phenolic content. The increase in antioxidant activity due to low temperature storage was always higher in non-cured roots than cured roots (Figure 5.2). However, a significant difference was observed only

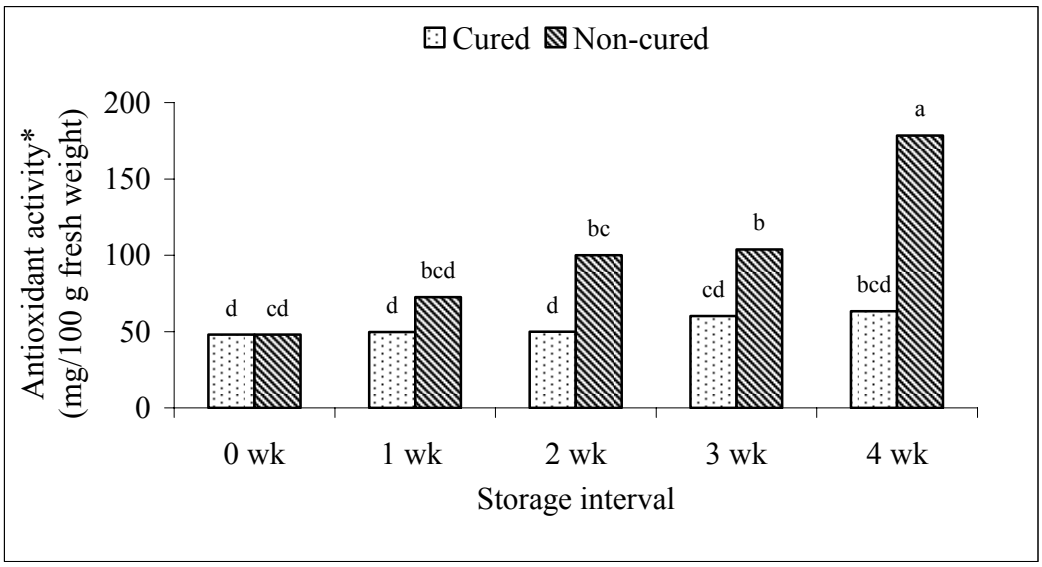

Figure 5.2. Antioxidant activity of cured and non-cured sweetpotato roots at different intervals of storage at 5 °C. Mean values with different letters are significantly different at $P<0.05$ (Tukey's test). *Data expressed as mg of Trolox equivalents per 100 g of fresh weight.

after 2 weeks of 5 °C storage. Antioxidant activity in freshly harvested roots was 48.5 mg Trolox equivalent/100 g fresh weight. After 4 weeks of 5 °C storage, antioxidant activity of non-cured roots increased to 178.4 mg Trolox equivalent/100 g fresh weight compared to 63.3 mg Trolox equivalent/100 g fresh weight in cured roots. The antioxidant activity of extract from roots chilled for 4 weeks was significantly higher than all other treatments.

A comparison of different tissues revealed that skin tissue had the highest antioxidant activity irrespective of low temperature storage duration. The rate of increase in antioxidant activity in skin tissue was higher than in other tissue types. The antioxidant activity of freshly harvested root tissue was: skin (257.3 mg Trolox equivalent/100 g fresh tissue); cortex (77.2 mg Trolox equivalent/100 g fresh tissue); cambium (123.4 mg Trolox equivalent/100 g fresh tissue); pith (48.8 mg Trolox equivalent/100 g fresh tissue) (Table 5.2). Our results are consistent with other findings where sweetpotato outer skin tissue was shown to have a two- to three-fold higher antioxidant activity than the inner ptih tissue (Troung et al., 2004; Cevallos-Casalas and Cisneros-Zevallos, 2003). Troung et al. (2004) reported that antioxidant activity of outer (5 mm) layer and inner tissue of different sweetpotato cultivars ranged from 75 to 175 mg and 50 to 70 mg Trolox equivalent/100g fresh weight. Our results indicated that none of the tissues showed any

Table 5.2. Antioxidant activity in different tissues of non-cured sweetpotato roots at different intervals of storage at 5 °C, including transfer to ambient temperature (22 °C) for an additional 3 days (d).

Storage interval	Antioxidant activity* (mg/100 g fresh weight)			
	Skin	Cortex	Cambium	Pith
0 wk	257.3[c]	77.2[a]	123.4[b]	48.8[a]
2 wk	303.5[c]	98.1[a]	121.8[b]	45.2[a]
2 wk + 3 d	480.8[b]	88.5[a]	124.3[b]	48.7[a]
4 wk	515.3[b]	102.6[a]	232.7[ab]	55.0[a]
4 wk + 3 d	805.2[a]	197.4[a]	270.2[a]	65.7[a]

Means with the same letters in a column are not significantly different at $P<0.05$ (Tukey's test). *Data expressed as mg of Trolox equivalents per 100 g of fresh weight.

significant increase in antioxidant activity during first 2 weeks of 5 °C storage. A three day exposure to warmer temperature after 2 weeks of 5 °C storage resulted in an increase in antioxidant activity in all tissues except the pith. The skin tissue had the highest antioxidant activity (805.2 mg Trolox equivalent/100 g fresh weight) after 4 weeks of 5 °C storage followed by a three day exposure to warmer temperature. The changes in

antioxidant activity of sweetpotato root tissue paralleled that of the total phenolic content. A strong correlation coefficient (r^2=0.99) existed between total phenolic content and antioxidant activity of non-cured roots stored at 5 °C (Figure 5.3).

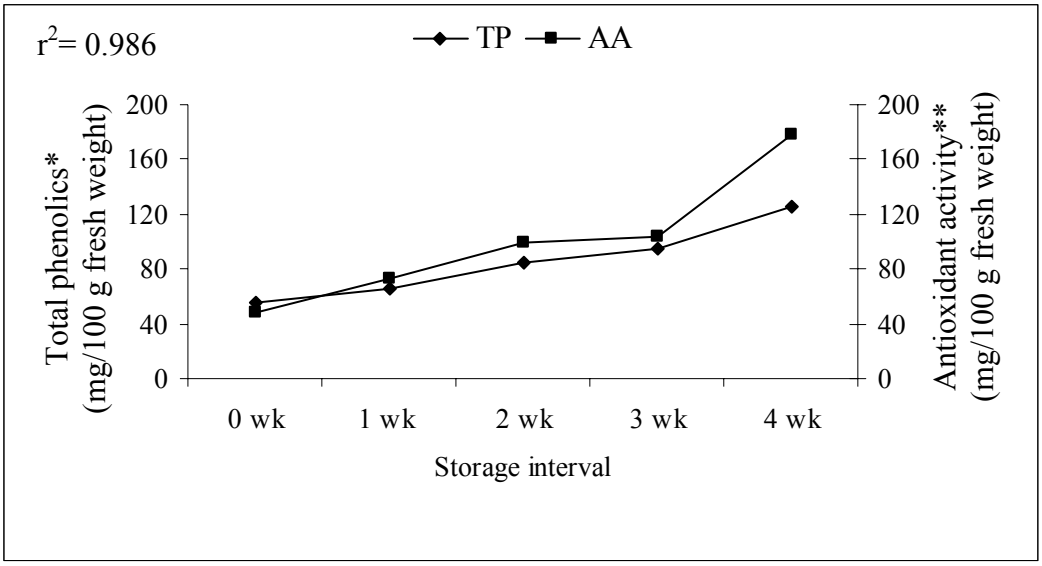

Figure 5.3. Correlation between total phenolics (TP) and antioxidant activity (AA) in non-cured sweetpotato roots during 4 weeks of storage at 5 °C. *Data expressed as mg of chlorogenic acid equivalents per 100 g of fresh weight. **Data expressed as mg of Trolox equivalents per 100 g of fresh weight.

Individual Phenolic Acids

Individual phenolic acids in sweetpotato tissues increased in roots exposed to low temperature storage. The individual phenolic acids identified in freshly harvested sweetpotato root tissues were: ChlA; CafA; 4,5-diCQA; 3,5-diCQA; and 3,4-diCQA. The major phenolic acid quantified was chlorogenic acid, with an average content ranging from 5.3 mg/100 g in fresh pith tissue to 18.3 mg/100 g in fresh skin tissue. All individual phenolic acids, except caffeic acid, increased two- to six-fold during low temperature storage, depending on the tissue location and low temperature storage duration (Tables 5.3-5.7). A further two-fold increase in dicaffeoylquinic acids (4,5-diCQA; 3,5-diCQA; and 3,4-diCQA) was observed when roots were transferred to

warmer temperature for 3 days. Consistent with our results, an increase in chlorogenic acid content in 'Porto Rico' sweetpotatoes had been previously reported (Lieberman et al., 1959). A further increase in individual phenolic acid concentration was observed in roots exposed to 3 days of warmer temperature after 4 weeks of chilling. However, caffeic acid content was much higher in skin tissue and showed no significant change with low temperature exposure. Our results agree with Harrison et al. (2003) who suggested the high periderm caffeic content may be associated with a chemical defense mechanism in some sweetpotato genotypes.

In general, the rate of increase in chlorogenic acid and dicaffeoylquinic acids content was highest in the skin tissue followed by the cambium tissue. The pith tissue had the lowest rate of increase in individual phenolic acids. Transversely sliced sweetpotato roots exposed to air showed more discoloration in the cambium ring than in the cortex and pith tissue. This is probably due to the higher phenolic acid content in the cambium tissue. Further identification of the specific phenolic compounds responsible for chill-induced discoloration needs to be investigated. Chlorogenic acid was found to be the most abundant phenolic acid in apples, but catechin was the main contributor to discoloration (Murata et al., 1995).

The two unidentified peaks immediately after and before chlorogenic acid were assumed to be isomers of chlorogenic acid, but this needs confirmation. The trend of increase in most of the individual phenolic acids with increasing duration of low temperature exposure paralleled the changes in total phenolic content.

Table 5.3. Chlorogenic acid content in different tissues of non-cured roots sweetpotato roots at different intervals of storage at 5 °C, including transfer to ambient temperature (22 °C) for an additional 3 days (d).

Storage interval	Chlorogenic acid (mg/100 g fresh weight)			
	Skin	Cortex	Cambium	Pith
0 wk	18.3[d]	10.1[b]	10.2[b]	5.3[a]
2 wk	25.2[d]	17.3[b]	18.1[b]	6.7[a]
2 wk + 3 d	61.6[bc]	24.2[ab]	19.8[b]	10.7[a]
4 wk	76.1[ab]	23.7[ab]	33.6[ab]	9.9[a]
4 wk + 3 d	93.4[a]	42.0[a]	59.1[a]	9.3[a]

Means with the same letters in a column are not significantly different at P<0.05 (Tukey's test).

Table 5.4. Caffeic acid content in different tissues of non-cured roots sweetpotato roots at different intervals of storage at 5 °C, including transfer to ambient temperature (22 °C) for an additional 3 days (d).

Storage interval	Caffeic acid (mg/100 g fresh weight)			
	Skin	Cortex	Cambium	Pith
0 wk	9.2[a]	0.4[a]	0.3[b]	0.2[a]
2 wk	10.7[a]	0.4[a]	0.4[b]	0.2[a]
2 wk + 3 d	8.6[a]	0.4[a]	0.5[b]	0.4[a]
4 wk	5.8[a]	0.7[a]	1.2[ab]	0.4[a]
4 wk + 3 d	6.7[a]	1.3[a]	3.9[a]	0.4[a]

Means with the same letters in a column are not significantly different at P<0.05 (Tukey's test).

Table 5.5. 4,5-dicaffeoylquinic acid content in different tissues of non-cured roots sweetpotato roots at different intervals of storage at 5 °C, including transfer to ambient temperature (22 °C) for an additional 3 days (d).

Storage interval	4,5-dicaffeoylquinic acid (mg/100 g fresh weight)			
	Skin	Cortex	Cambium	Pith
0 wk	3.2[b]	0.5[b]	1.2[b]	0.4[a]
2 wk	3.0[b]	0.6[b]	1.3[b]	0.4[a]
2 wk + 3 d	10.1[b]	1.1[ab]	1.6[b]	1.5[a]
4 wk	11.0[ab]	1.1[ab]	2.9[ab]	0.7[a]
4 wk + 3 d	18.2[a]	3.8[a]	13.7[a]	1.1[a]

Means with the same letters in a column are not significantly different at P<0.05 (Tukey's test).

Table 5.6. 3,5-dicaffeoylquinic acid content in different tissues of non-cured roots sweetpotato roots at different intervals of storage at 5 °C, including transfer to ambient temperature (22 °C) for an additional 3 days (d).

Storage interval	3,5-dicaffeoylquinic acid (mg/100 g fresh weight)			
	Skin	Cortex	Cambium	Pith
0 wk	5.4[b]	1.3[b]	3.4[b]	0.8[a]
2 wk	7.9[b]	4.1[b]	7.3[b]	1.3[a]
2 wk + 3 d	25.2[b]	4.9[b]	6.2[b]	3.8[a]
4 wk	25.5[b]	3.8[b]	8.2[b]	1.5[a]
4 wk + 3 d	95.9[a]	23.1[a]	55.0[a]	3.2[a]

Means with the same letters in a column are not significantly different at $P<0.05$ (Tukey's test).

Table 5.7. 3,4-dicaffeoylquinic acid content in different tissues of non-cured roots sweetpotato roots at different intervals of storage at 5 °C, including transfer to ambient temperature (22 °C) for an additional 3 days (d).

Storage interval	3,4-dicaffeoylquinic acid (mg/100 g fresh weight)			
	Skin	Cortex	Cambium	Pith
0 wk	7.2[c]	2.8[b]	6.6[b]	1.8[a]
2 wk	6.5[c]	3.9[b]	7.8[b]	1.5[a]
2 wk + 3 d	19.9[bc]	4.2[b]	5.7[b]	3.9[a]
4 wk	35.0[b]	6.7[b]	14.0[b]	2.6[a]
4 wk + 3 d	73.6[a]	22.1[a]	65.9[a]	4.0[a]

Means with the same letters in a column are not significantly different at $P<0.05$ (Tukey's test).

CONCLUSIONS

The increase in total phenolic content in low temperature stored sweetpotatoes was higher in non-cured roots than in cured roots. Exposure to warmer temperature after low temperature storage resulted in an accelerated buildup of phenolic compounds. Skin tissue had the highest concentration of total phenolics, individual phenolic acids, and highest antioxidant activity. Chlorogenic acid and caffeic acid were the most and least abundant phenolic acids in sweetpotato roots, respectively, except in skin tissue of freshly harvested roots where caffeic acid was not the least concentrated, but was secondary to chlorogenic acid. A limited time exposure to low temperature may enhance the

nutraceutical value of sweetpotatoes without any loss of marketable quality.

Sweetpotatoes exposed to low temperature stress for extended periods may be targeted by the functional food industry as a concentrated source of phenolic antioxidants.

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CHAPTER 6. PHENOLIC COMPOSITION AND ANTIOXIDANT ACTIVITY OF MINIMALLY PROCESSED SWEETPOTATOES

INTRODUCTION

Minimally processed fruits and vegetables are popular value-added convenience foods widely available worldwide. Consumption of fresh-cut produce has grown at a rapid pace during the last 10 years and new product development is still occurring (Garrett, 2002). Fresh-cut products are substituting for frozen items in certain cases. A wide variety of fresh-cut products are now commercially available for direct consumer use or for further processing by restaurants and fast-food chains. Many consumers are looking for convenience, high quality, and healthy choice products.

Controlled abiotic stress treatments such as minimal processing may be used to enhance the health benefits from fresh-cut produce via an increase in secondary metabolites, such as phenolics (Cisneros-Zevallos, 2003). An increase in total phenolic content and antioxidant activity was observed in minimally processed carrots, potato, jicama roots, escarole, and radicchio (Aquino-Bolanos et al., 2000; Cantos et al., 2002; Cisneros-Zevallos, 2003; Di Venere et al., 2005). The phenolic compounds act as antioxidant or free radical scavengers and may help to reduce the risks of certain types of cancer, diabetes, and cardiovascular disease (Scalbert et al., 2005). The antioxidant properties of phenolic compounds have been implicated in suppressing hepatoma invasion (Yagasaki et al., 2000), and human immunodeficiency virus (HIV) replication (Mahmood et al., 1993). The phenolic acids such as chlorogenic acid (ChlA); 3,5-dicaffeoylquinic acid (3,5-diCQA); 3,4-dicaffeoylquinic acid (3,4-diCQA); and 4,5-dicaffeoylquinic acid (4,5-diCQA) extracted from steamed sweetpotato suppressed melanogenesis in mice (Shimozono et al., 1996). The potential chemopreventive

properties of water extract from baked sweetpotatoes had been reported recently (Rabah et al., 2004).

The sweetpotato [*Ipomoea batatas* (L.) Lam.] root is a major staple food widely consumed in developing countries and ranks as the seventh most important food crop in the world (CIP, 2006). Like other vegetables, sweetpotato can also be minimally processed and consumed as fresh-cut product. Ready-to-use sweetpotato forms such as shredded, sliced or French-fry cuts may decrease preparation time for use in restaurants and other food service facilities. Sweetpotato roots have been reported to contain phenolic antioxidant compounds (Walter et al., 1979; Oki et al., 2002; Huang et al., 2004). Abiotic stress, such as wounding of the tissue, may further increase the phenolic content and nutraceutical value of sweetpotato roots. Kojima and Uritani (1973) reported that slicing of sweetpotato roots resulted in marked polyphenol production. However, no information exists pertaining to the antioxidant activity in different types of fresh-cut sweetpotato products. The objective of this study was to quantify the phenolic composition and antioxidant activity changes during storage of different style cut sweetpotatoes.

MATERIALS AND METHODS

Reagents

Chlorogenic acid and caffeic acid standards, Folin-Denis reagent and DPPH (1, 1-diphenyl-2-picrylhydazyl) reagent were purchased from Sigma-Aldrich (St. Louis, MO). Standards of three isomers of isochlorogenic acid were kindly provided by Dr. Makoto Yoshimoto (Department of Upland Farming, Kyushu National Agricultural Experiment Station, Miyakonojo, Miyazaki, Japan).

Tissue Preparation

Sweetpotato roots cv. 'Beauregard' were peeled with an abrasive peeler and minimally processed into shreds (approximately 2 × 20 mm), slices (5 mm thick), and French-fry cuts (7 × 10 mm). A manual food processor was used for shredding, whereas the French-fry cut and slices were prepared using a Bron Coucke mandoline (Model No. 20638CHB, Discount Natural Foods Inc., Hooksett, NH). The fresh-cut material was sanitized by dipping in a 150 mg/l chlorine solution (pH 6.5) for 1 minute. The sanitized material was spin-dried using a salad spinner (Item No. 32480, Oxo International, New York, NY) and a random sample of 100 g from each cut was heat sealed in 13 × 20 cm polyethylene bags. The bags of fresh-cut material were stored at two different temperatures, 0 and 5 °C, and analyzed after 4 and 8 days of storage. Sweetpotatoes analyzed immediately after cutting (0 days of storage) served as the control.

Headspace Gas Composition

The gas composition inside the bags was monitored daily using a Model 6600 oxygen/carbon dioxide analyzer (Illinois Instruments, Ingleside, IL). An adhesive septum was affixed to the plastic bag to avoid any leakage after sampling. To make the gaseous mixture homogenous, the bags were gently shaken prior to gas analysis.

Tissue Extraction

Following removal from storage, randomly selected tissue from each bag was lyophilized and powdered using a mortar and pestle. Exactly 0.5 g of tissue was placed in a 15 ml Falcon centrifuge tube. Approximately 8 ml of 80% methanol was added to the centrifuge tube. The tubes were capped and immersed in a water bath at 80 °C for 10 minutes. After vigorously shaking the heated samples manually for 30 seconds, the tubes

were cooled and centrifuged at 4,500 g for 15 minutes. The final volume of clear supernatant was made to 10 ml with 80% methanol and analyzed for total phenolic content, individual phenolic acids, and antioxidant activity.

Total Phenolics

Total phenolic content was determined by a modification of the Folin-Denis method (Swain and Hillis, 1959). Exactly 0.5 ml of supernatant was placed in a 25 ml test tube and mixed with 8 ml of Megapure water (Barnstead MP-12A, Haverhill, MA) followed by the addition of 0.5 ml of Folin-Denis reagent. After 3 minutes, 1 ml of 1N Na_2CO_3 was added and the solution was allowed to stand for 2 hours at ambient temperature (~22 °C). Absorbance of the resulting blue complex was measured at 750 nm using a Lambda 35 UV/Vis spectrophotometer (PerkinElmer Instruments, Norwalk, CT). A standard curve of chlorogenic acid (50-300 µg/ml concentration) was plotted. The total phenolic content was expressed as mg ChlA equivalent/g dry weight.

Antioxidant Activity

The antioxidant activity was measured according to Brand-Williams et al. (1995), with slight modifications. DPPH (1, 1-diphenyl-2-picrylhydazyl) was used as the source of free radicals. The absorbance of free radicals at 520 nm disappears upon their reduction by an antioxidant. In this study, Trolox (6-hydroxy-2, 5, 7, 8-tetramethyl-chroman-2-carboxylic acid) was used as the standard antioxidant compound. An aliquot (300 µl) of sweetpotato extract was placed in a 1.5 ml amber colored centrifuge tube. Then 600 µl of 0.1 M 2-(N-morpholine) ethanesulfonic acid (MES) (pH 6.0) and 300 µl of 0.4 mM of DPPH solution were added to the centrifuge tube. The resultant mixture was shaken and held in the dark for 2 minutes. The decrease in DPPH absorbance was

measured using a Lambda 35 UV/Vis spectrophotometer (PerkinElmer Instruments, Norwalk, CT) at 520 nm. The sweetpotato extract replaced with 80% methanol was taken as control. The spectrophotometer was calibrated using 80% methanol as a blank. The antioxidant activity was calculated from a standard curve made with known concentrations of Trolox and expressed in terms of mg Trolox equivalent/g dry weight.

Individual Phenolic Acids

Separation of individual phenolic acids was accomplished using reversed-phase HPLC (high performance liquid chromatography) with a Gemini C18, 5 µm, 4.6 × 250 mm (Phenomenex, Torrance, CA) column. An aliquot of the supernatant was filtered through a 0.45 µm Nylaflo membrane filter (Pall Corp., East Hills, NY). A sample volume of 20 µl was injected onto the column using a Waters 717 autosampler connected to a Waters 600E pump (Waters Corp., Milford, MA). Phenolic acids were eluted using a mobile phase consisting 1% (v/v) formic acid in aqueous solution: acetonitrile: 2-propanol (70:22:8), pH 2.5 with an isocratic flow rate of 0.75 ml/min. The peaks detected were identified and quantified by comparing the retention time and peak area to that of known standards. Quantification was based on absorbance at 320 nm using a Waters 2487 dual wavelength UV absorbance detector.

Statistical Analysis

A completely randomized design was used. Each bag was considered as one replication with five replications per treatment. Data was analyzed by SAS procedure MIXED (SAS Institute Inc., Cary, NC) and means were separated using Tukey's test ($P<0.05$).

RESULTS AND DISCUSSION

Headspace Gas Composition

The changes in O_2 and CO_2 levels with time inside the packages of fresh-cut sweetpotatoes are shown in Figures 6.1 and 6.2. Irrespective of the style of cut, the O_2 and CO_2 levels in sweetpotatoes stored at 0 °C did not change significantly during the 8 day storage period. However, the types of cut or injury level significantly affected the

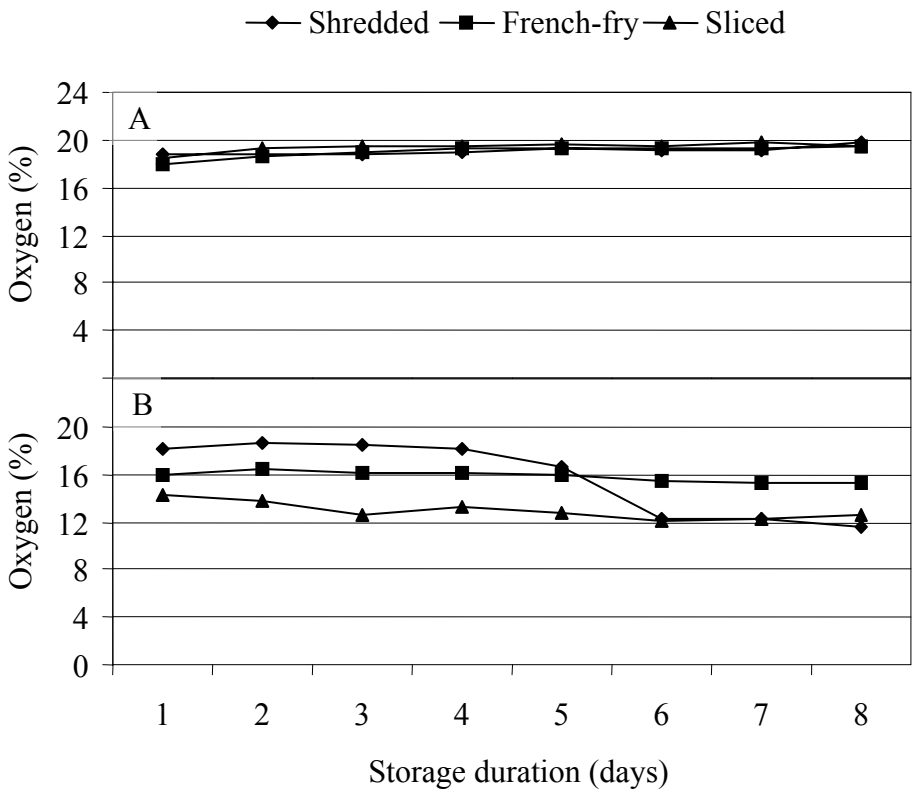

Figure 6.1. Changes in headspace O_2 levels in minimally processed sweetpotatoes stored during storage at 0 °C (A) and 5 °C (B). Each data point represents the mean value of 5 replications.

headspace gas composition in bags stored at 5 °C. After 4 days of storage at 5 °C, the O_2 levels were highest in the shredded and lowest in the sliced cut, while CO_2 levels followed the opposite trend. A sharp decline in O_2 level with a corresponding increase in CO_2 level was observed in the shredded cut after 4 days of storage at 5 °C. The O_2 levels

in the bags stored for 8 days were sufficient to avoid anaerobic respiration and no decay or detectable off-flavor was observed in any of the cuts. Consistent with our results, previous reports indicated respiration rate of fresh cut jicama roots stored at 5 and 10 °C increased substantially after 4 and 2 days, respectively (Aquino-Bolanos et al., 2000).

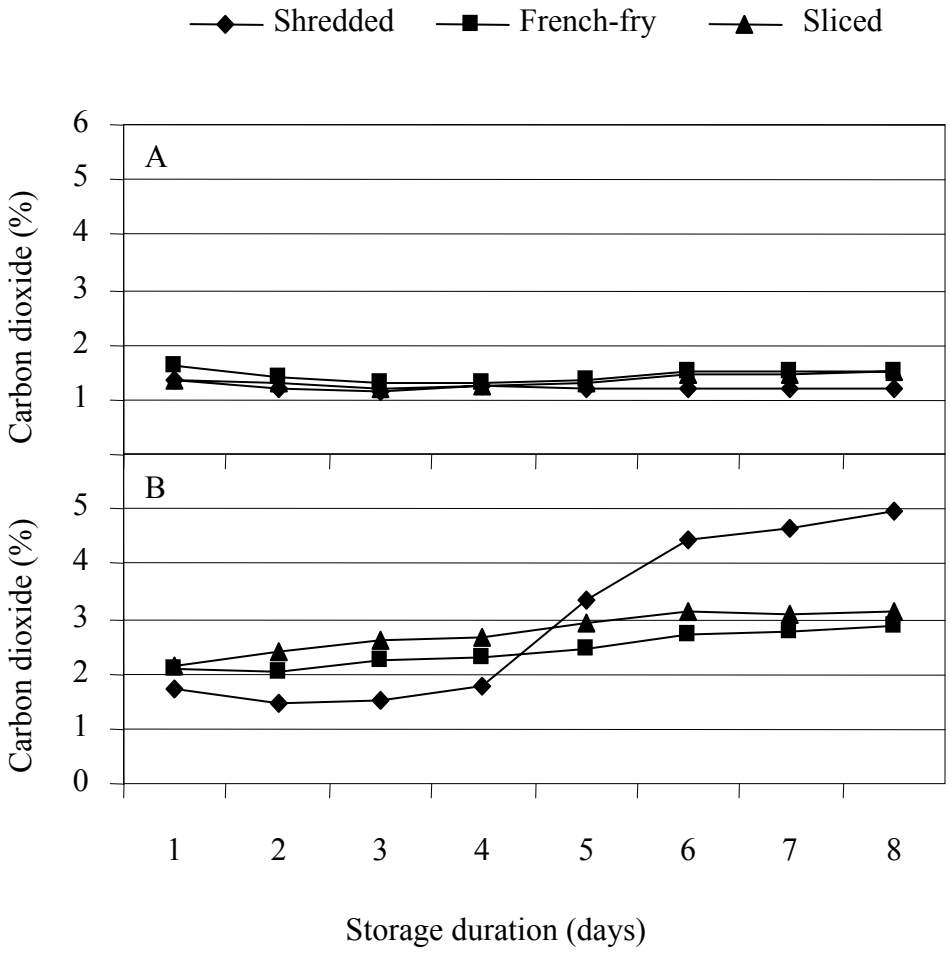

Figure 6.2. Changes in headspace CO_2 levels in minimally processed sweetpotatoes stored during storage at 0 °C (A) and 5 °C (B). Each data point represents the mean value of 5 replications.

Total Phenolics

The initial total phenolic content in sweetpotato root tissue immediately after cutting (0 days of storage) was 1.3 mg/g dry weight. Minimal processing of sweetpotatoes followed by low temperature storage resulted in an increase in total

phenolic content (Table 6.1). In general, sliced cut sweetpotatoes had a significantly higher total phenolic content than shredded cut. However, the rates of increase in total phenolic content with duration of storage depend on storage temperature. The storage of minimally processed sweetpotatoes at 5 °C resulted in higher total phenolic content than

Table 6.1. Total phenolics and antioxidant activity in minimally processed sweetpotatoes stored at 0 and 5 °C for 4 and 8 days.

Processing type	Storage temperature	Storage (days)	Total phenolics*	Antioxidant activity**
Control		0	1.3^{d}	0.8^{d}
Shredded	0 °C	4	1.3^{d}	0.8^{d}
		8	1.3^{d}	0.8^{d}
	5 °C	4	1.4^{cd}	1.1^{c}
		8	2.2^{a}	1.8^{b}
French-fry cut	0 °C	4	1.4^{cd}	1.0^{c}
		8	1.5^{cd}	1.1^{c}
	5 °C	4	1.7^{bc}	1.3^{c}
		8	2.3^{a}	2.3^{a}
Sliced	0 °C	4	1.5^{bc}	1.2^{c}
		8	1.6^{bc}	1.1^{c}
	5 °C	4	1.8^{b}	1.6^{b}
		8	2.4^{a}	2.4^{a}

Means with the same letters in a column are not significantly different at $P<0.05$ (Tukey's test). *Data expressed as mg of chlorogenic acid equivalents per g of dry weight. **Data expressed as mg of Trolox equivalents per g of dry weight.

at 0 °C. The total phenolic content in sliced tissue (2.4 mg/g dry weight) after 8 days of storage at 5 °C was approximately two-fold higher than the total phenolic content immediately after cutting (1.3 mg/g dry weight). Results for total phenolic content in minimally processed sweetpotatoes were consistent with previous reports on other crops. Total phenolic content increased when fresh-cut escarole and radicchio were stored for 9 days at 4 °C (Di Venere et al., 2005). The increase in total phenolic content in fresh-cut jicama root tissue was associated with phenylalanine ammonia-lyase (PAL) activity, which increased significantly at 10 °C (Aquino-Bolanos et al., 2000). It has been suggested that the induction of phenolic compounds serves as a chemical defense

mechanism to resist pathogen attack after tissue damage (Dixon and Paiva, 1995). Although phenolic compounds increased after 8 days of storage at 5 °C, no tissue browning or off-flavor was observed and the products were considered to be marketable. Previously, no significant correlation was found between the degree of browning and total phenolic content of fresh-cut potatoes (Cantos et al., 2002). However, the increased total phenolic content during storage of minimally processed carrots may have contributed to the chemical defense against pathogen attack (Chubey and Nylund, 1969).

Antioxidant Activity

The trend of change in antioxidant activity paralleled that of total phenolic content (Table 6.1). The initial antioxidant activity of sweetpotato roots immediately after cutting (0 days of storage) was 0.78 mg Trolox equivalent/g dry weight. The storage of minimally processed sweetpotatoes resulted in increased antioxidant activity. During the storage period of 8 days, sliced cut and shredded cut had the highest and lowest antioxidant activities, respectively. Irrespective of the style of cut and duration of storage, the minimally processed roots stored at 5 °C showed higher antioxidant activity than roots stored at 0 °C. At 5 °C, the fresh cut sweetpotatoes stored for 8 days had significantly higher antioxidant activity than after 4 days storage. The highest antioxidant activity (2.4 mg Trolox equivalent/g dry weight) in sliced tissue stored for 8 days at 5 °C was approximately three-fold higher than the initial antioxidant activity (0.8 mg Trolox equivalent/g dry weight) of sweetpotatoes analyzed immediately after cutting. The accumulation of phenolic compounds may be a key factor responsible for increased antioxidant activity of minimally processed sweetpotatoes. Thus, minimally processing of sweetpotatoes followed by storage at specific temperature may enhance the antioxidant

properties of sweetpotatoes. A recent study indicated that increased antioxidant potential of fresh-cut escarole and radicchio stored for 9 days at 4°C was associated with increased total phenolic content (Di Venere et al., 2005).

Individual Phenolic Acids

The quantitative results on the pattern of changes in individual phenolic acid content during storage of minimally processed sweetpotatoes are presented in Table 6.2. The most abundant phenolic acid in sweeetpotato roots was chlorogenic acid followed by 3,5-dicaffeoylquinic acid. The other identified phenolic acids such as caffeic; 4,5-dicaffeoylquinic; and 3,4-dicaffeoylquinic acids were present in very low amounts. In general, the content of the five identified phenolic acids increased many fold, reaching maximal levels on the eighth day of storage at 5 °C. However, storage temperature and style of cut significantly affect the accumulation of phenolic acids. The accumulation of phenolic acids at 5 °C was always higher than at 0 °C. Tomas-Barberan et al. (1997) reported that due to higher metabolism, the wound induced synthesis of caffeic acid derivatives in lettuce was higher at 10 than at 5 °C.

After 8 days of storage at 5 °C, the content of all phenolic acids, except 4,5-dicaffeoylquinic acid, was significantly higher than the initial phenolic acid content in sweetpotatoes analyzed immediately after cutting. Chlorogenic acid content in sliced cut stored at 5 °C for 8 days was six-fold higher than immediately after cutting. Consistent with our results, a dramatic increase in the chlorogenic acid content occurred in fresh-cut potato strips and shredded carrots stored at 4 °C (Tudela et al., 2002; Babic et al., 1993). At the end of the storage period, sliced tissue had significantly higher amount of chlorogenic acid than other style cuts. However, the content of 3,4-dicaffeoylquinic acid

Table 6.2. Individual phenolic acid accumulation during storage of minimally processed sweetpotatoes.

Phenolic acid	Control	Shredded				French-fry cut				Sliced			
	0 days	4 days		8 days		4 days		8 days		4 days		8 days	
		0 °C	5 °C	0 °C	5 °C	0 °C	5 °C	0 °C	5 °C	0 °C	5 °C	0 °C	5 °C
ChlA	88.3^{g}	75.4^{g}	150.6^{e}	97.9^{fg}	306.1^{c}	93.7^{fg}	205.9^{d}	106.9^{efg}	414.1^{b}	104.3^{efg}	261.0^{c}	144.1^{ef}	539.9^{a}
CafA	1.4^{f}	2.0^{ef}	5.8^{c}	3.4^{def}	1.2^{f}	$3.0d^{ef}$	8.2^{b}	3.8^{cde}	14.5^{a}	3.2^{def}	8.4^{b}	4.8^{cd}	15.8^{a}
4,5-diCQA	5.5^{ab}	2.6^{c}	3.9^{bc}	3.7^{bc}	5.0^{abc}	2.5^{c}	4.5^{abc}	2.6^{c}	5.0^{abc}	3.2^{bc}	6.1^{ab}	7.1^{a}	5.9^{ab}
3,5-diCQA	68.6^{d}	57.2^{d}	91.0^{bcd}	71.9^{cd}	184.1^{a}	80.8^{bcd}	110.0^{b}	88.1^{bcd}	217.4^{a}	75.6^{bcd}	108.5^{bc}	94.1^{bcd}	210.8^{a}
3,4-diCQA	4.3^{e}	3.0^{e}	14.1^{b}	3.3^{e}	17.3^{a}	4.4^{cde}	7.1^{c}	3.6^{e}	12.3^{b}	3.9^{de}	6.3^{cd}	5^{cde}	12.2^{b}

Data expressed as µg of individual phenolic acid per g of dry weight. Mean values (n=5) with same letters within a row are not significantly different at $P<0.05$ (Tukey's test). ChlA: chlorogenic acid; CafA: caffeic acid; diCQA: dicaffeoylquinic acid.

in the shredded cut was significantly higher than other cuts. This study indicated that specific type or level of tissue injury may induce the production of specific types of phenolic acids. More information on health benefits imparted by individual phenolic acids may be helpful in marketing fresh-cut sweetpotatoes as a functional food.

CONCLUSIONS

Minimal processing of sweetpotatoes and subsequent storage can induce the accumulation of phenolic compounds. Sliced cut sweetpotatoes had a higher total phenolic and antioxidant activity than the other cuts. The increased phenolic content may enhance the nutraceutical value and health beneficial properties of sweetpotatoes. In addition to increased nutraceutical value, no loss in marketable quality was observed during storage of fresh-cut sweetpotatoes for 8 days at 0 °C or 5 °C. This information may be useful to the food industry in targeting fresh-cut sweetpotato as a convenience food with increased health benefits. Further research on the effect of internal O_2 and CO_2 content on sweetpotato phenolics and antioxidant activity may help to optimize the conditions which favor a high nutraceutical value and longer shelf life of fresh-cut sweetpotatoes.

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CHAPTER 7. PHENOLIC COMPOSITION AND ANTIOXIDANT ACTIVITY OF SWEETPOTATO CULTIVARS

INTRODUCTION

The role of polyphenols in preventing many chronic diseases including cancer, cardiovascular diseases, and diabetes has been well documented (Scalbert et al., 2005). Recent interest in phenolics and antioxidant compounds has prompted research to explore the health beneficial properties of many fruits and vegetables. Sweetpotato, an important food crop of the world, has been reported to be a good source of phenolics and antioxidants (Walter et al., 1979; Hayase and Kato, 1984). Recent studies have revealed the potential chemopreventive properties of sweetpotato extracts (Rabah et al., 2004; Shimozono et al., 1996). Most of the commercially grown sweetpotato cultivars are orange-fleshed or white-fleshed; however, purple-fleshed cultivars are used as a source of natural food colorant (Yoshinaga et al., 1999. Purple-fleshed sweetpotatoes were reported to have higher antioxidant properties than orange or white-fleshed cultivars (Furuta et al., 1998). In addition to anthocyanins, phenolic acids (i.e. chlorogenic and dicaffeoylquinic acids) contribute to the antioxidant activity and other heath beneficial effects of purple-fleshed cultivars (Oki et al., 2002).

Sweetpotato cultivars of different flesh color may differ in their phenolic composition and antioxidant properties. In addition, cultivars with the same flesh color may differ in total phenolic content, individual phenolic acid profile, and antioxidant activity. Among six orange-fleshed cultivars analyzed, the total and individual phenolic acid content was highest in ‘Australian Canner’ and lowest in ‘Centennial’ (Walter and Purcell, 1979). Four isomers of caffeoylquinic acid were reported to be present in fourteen sweetpotato cultivars and breeding lines, although they were not quantified

(Thompson, 1981). Harrison et al. (2003) reported caffeic acid content varied greatly among 24 sweetpotato cultivars and breeding lines. Despite the previous studies, there is limited information available on phenolic composition and antioxidant properties of the major sweetpotato cultivars grown and marketed in the U.S. and E.U. (European Union). This study was initiated to compare the phenolic composition and antioxidant activity of commercially important sweetpotato cultivars and breeding lines.

MATERIALS AND METHODS

Reagents

Chlorogenic acid and caffeic acid standards, Folin-Denis reagent and DPPH (1, 1-diphenyl-2-picrylhydazyl) reagent were purchased from Sigma-Aldrich (St. Louis, MO). Standards of three isomers of dicaffeoylquinic acid (4,5-diCQA; 3,5-diCQA; 3,4-diCQA) were kindly provided by Dr. Makoto Yoshimoto (Department of Upland Farming, Kyushu National Agricultural Experiment Station, Miyakonojo, Miyazaki, Japan).

Plant Material

Sweetpotato cv. 'Beauregard' and breeding lines 9935 (orange-fleshed), 03-268 (orange-fleshed), 04-10 (white-fleshed) and 02-814 (purple-fleshed) were grown at the LSU AgCenter Burden Research Center at Baton Rouge, La, in 2005. An additional twelve most important commercial cultivars in the E.U. market were obtained from importers in January, 2006.

Tissue Preparation and Extraction

Randomly selected sound roots, approximately 300 g in weight, were washed and dried at ambient temperature (~22 °C) before tissue preparation. Roots were cut into half and grated manually. The grated tissue was immediately frozen at -45 °C, lyophilized,

and powdered for further extraction. Exactly 0.5 g of lyophilized tissue (root or leaf) was placed in a 15 ml Falcon centrifuge tube. Approximately 8 ml of 80% methanol was added to the centrifuge tube. The tubes were capped and immersed in a water bath at 80 °C for 10 minutes. After vigorously shaking the heated samples for 30 seconds, the tubes were cooled and centrifuged at 4,500 g for 15 minutes. The clear supernatant was decanted and brought to a final volume of 10 ml using 80% methanol and analyzed for total phenolic content, individual phenolic acids content and antioxidant activity.

Total Phenolics

Total phenolic content was determined by modification of the Folin-Denis method (Swain and Hillis 1959). Exactly 0.5 ml of supernatant was placed in a 25 ml test tube and mixed with 8 ml of Megapure water (Barnstead MP-12A, Haverhill, MA) followed by the addition of 0.5 ml of Folin-Denis reagent. After 3 minutes, 1 ml of 1 N Na_2CO_3 was added and the solution was allowed to stand for 2 hours at ambient temperature (~21 °C). Absorbance of the resulting blue complex was measured at 750 nm using a Lambda 35 UV/Vis spectrophotometer (PerkinElmer Instruments, Norwalk, CT) A standard curve of chlorogenic acid (50-300 µg/ml concentration) was plotted. The total phenolic content was expressed as mg chlorogenic acid equivalent/g dry weight.

Antioxidant Activity

The antioxidant activity was measured according to Brand-Williams et al. (1995), with slight modifications. DPPH (1, 1-diphenyl-2-picrylhydazyl) was used as the source of free radicals. The absorbance of free radicals at 520 nm disappears upon their reduction by an antioxidant. In this study, Trolox (6-hydroxy-2, 5, 7, 8-tetramethyl-chroman-2-carboxylic acid) was used as the standard antioxidant compound. An aliquot

(300 µl) with a suitable dilution of sweetpotato extract was placed in a 1.5 ml amber colored centrifuge tube. Exactly 600 µl of 0.1 M 2-(N-morpholine) ethanesulfonic acid (MES) (pH 6.0) and 300 µl of 0.4 mM of DPPH solution were added to the centrifuge tube. The resultant mixture was shaken and held in the dark for 2 minutes. The decrease in DPPH absorbance was measured at 520 nm using a Lambda 35 UV/Vis spectrophotometer (PerkinElmer Instruments, Norwalk, CT). For the control, the sweetpotato extract was replaced with 80% methanol. The 80% methanol solution without DPPH was used to calibrate the spectrophotometer. The antioxidant activity was calculated from a standard curve made with known concentrations of Trolox and expressed in terms of mg Trolox equivalent/g dry weight.

Individual Phenolic Acids

Separation of individual phenolic acids was accomplished using reversed-phase HPLC with a Gemini C18, 5 µm, 4.6 × 250 mm (Phenomenex, Torrance, CA) column. An aliquot of the supernatant was filtered through a 0.45 µm Nylaflo membrane filter (Pall Corp., East Hills, NY). A sample volume of 20 µl of was injected onto the column using a Waters 717 autosampler connected to a Waters 600E pump (Waters Corp., Milford, MA). Phenolic acids were eluted using a mobile phase consisting 1% (v/v) formic acid in aqueous solution: acetonitrile: 2-propanol (70:22:8), pH 2.5 with an isocratic flow rate of 0.75 ml/min. Peaks detected were identified and quantified by comparing the retention time and peak area to that of known standards. Quantification was based on absorbance at 320 nm using a Waters 2487 dual wavelength UV absorbance detector.

Statistical Analysis

A completely randomized design with five replications per cultivar was used. Each root was considered as one replication. Data was analyzed by SAS procedure MIXED (SAS Institute Inc., Cary, NC) and means were separated using Tukey's test ($P<0.05$).

RESULTS AND DISCUSSION

Total Phenolics and Antioxidant Activity

Our results indicated that cultivars and breeding lines differed considerably in total phenolic content and antioxidant activity (Table 7.1). The total phenolic content and antioxidant activity of different cultivars and breeding lines ranged from 1.1 to 4.7 mg/g dry weight and 1.0 to 4.6 mg/g dry weight, respectively. A purple-fleshed breeding line,

Table 7.1. Total phenolics and antioxidant activity of different sweetpotato cultivars and breeding lines.

Cultivar or Breeding line	Source	Flesh Color	Total phenolics*	Antioxidant activity**
02-814	Louisiana	Purple	4.7^{a}	4.6^{a}
Jamaican	Jamaica	White	4.1^{ab}	3.4^{b}
Abees	Egypt	White	3.7^{ab}	3.0^{bc}
Rubina	Israel	Orange	3.4^{bc}	2.5^{cde}
Bosbok	Honduras	White	3.4^{bcd}	1.3^{g}
Diane	California	Orange	3.0^{bcde}	3.6^{b}
California Organic	California	Orange	2.5^{cdef}	2.7^{bcd}
Uganda	Uganda	White	2.4^{cdef}	1.8^{efg}
9935	Louisiana	Orange	2.0^{defg}	2.0^{def}
Darby	California	Orange	1.9^{efg}	1.9^{efg}
03-268	Louisiana	Orange	1.9^{efg}	1.8^{fg}
Beauregard	Louisiana	Orange	1.8^{efg}	1.8^{efg}
Kumara	New Zealand	White	1.7^{fg}	1.4^{fg}
Bosbok	Brazil	White	1.7^{fg}	1.9^{def}
Golden Sweet	California	Cream	1.6^{g}	1.0^{g}
O'Henry	Louisiana	White	1.4^{fg}	1.3^{fg}
04-10	Louisiana	White	1.1^{g}	1.6^{g}

*Data expressed as mg of chlorogenic acid equivalents per g of dry weight. **Data expressed as mg of Trolox equivalents per g of dry weight. Mean values (n=5) with the same letters in a column are not significantly different at $P<0.05$ (Tukey's test).

‘02-814’, had the highest total phenolic content, followed by a Jamaican grown white-fleshed cultivar. However, the antioxidant activity of the California-grown orange-fleshed cultivar ‘Diane’ was next highest to the purple-fleshed breeding line. A white-fleshed breeding line, ‘04-10’, and the California-grown cultivar ‘Golden Sweet’ contained the least amounts of total phenolic content and antioxidant activity, respectively. In general, there was a trend of increased antioxidant activity with increased total phenolic content.

In agreement with our results, Furuta et al. (1998) indicated purple-fleshed sweetpotatoes had higher antioxidant activity than orange- or white-fleshed cultivars. The high antioxidant activity of purple-fleshed sweetpotato cultivars was due to both anthocyanin and phenolic acids (Oki et al., 2002). This study indicated that sweetpotato cultivars vary widely in their antioxidant values and potential use as functional foods. The information generated from this research may assist breeders interested in increasing the health beneficial properties of a sweetpotato cultivar while ensuring the specific taste and flesh color preferred by the consumer. The contribution of phenolic compounds to sweetpotato taste needs to be further investigated.

Individual Phenolic Acids

Five individual phenolic acids were identified in the sweetpotato genotypes by comparing the retention time with those of known standards. Those included chlorogenic acid (ChlA), caffeic acid (CafA), 4,5-dicaffeoylquinic acid (4,5-diCQA), 3,5-dicaffeoylquinic acid (3,5-diCQA), and 3,4-dicaffeoylquinic acid (3,4-diCQA). Two unidentified peaks which eluted immediately before and after the chlorogenic acid peak were tentatively identified as isomers of chlorogenic acid (neochlorogenic and

cryptochlorogenic acid). Our results indicated significant differences exist in individual phenolic acid content among sweetpotato cultivars and breeding lines (Table 7.2). In general, chlorogenic and 3,5-dicaffeoylquinic acid were the two most abundant phenolic acids in all cultivars and breeding lines. However, the phenolic profiles of California-

Table 7.2. Individual phenolic acid content of different sweetpotato cultivars and breeding lines.

Cultivar or Breeding line	Source	Flesh color	ChlA	CafA	4,5-diCQA	3,5-diCQA	3,4-diCQA
			μg/g dry weight				
02-814	Louisiana	Purple	231.6^{bc}	20.5^{a}	284.4^{a}	485.6^{a}	125.6^{a}
Jamaican	Jamaica	White	422.4^{a}	6.2b^{cd}	25.3^{bcd}	431.6^{a}	25.8^{b}
Abees	Egypt	White	183.7^{cdef}	0.5^{d}	12.4b^{cd}	197.7^{bcd}	13.3^{b}
Rubina	Israel	Orange	181.3^{cde}	4.7^{cd}	16.0b^{cd}	268.4^{b}	10.8^{b}
Bosbok	Honduras	White	36.6^{g}	0.4^{d}	4.6^{cd}	46.0^{d}	0.7^{b}
Diane	California	Orange	357.8^{ab}	13.4^{ab}	17.2^{bcd}	177.5^{bcd}	12.3^{b}
California Organic	California	Orange	167.5^{cdef}	7.2^{bcd}	9.2^{bcd}	85.1^{cd}	1.9^{b}
Uganda	Uganda	White	168.0^{cdef}	1.4^{d}	9.9^{bcd}	205.4^{bc}	7.8^{b}
9935	Louisiana	Orange	169.8^{cdef}	9.4^{bc}	9.4^{bcd}	151.3^{bcd}	5.6^{b}
Darby	California	Orange	113.4^{cdefg}	5.2^{cd}	10.3^{bcd}	56.3^{cd}	5.9^{b}
03-268	Louisiana	Orange	197.9^{cd}	12.5^{b}	10.5^{bcd}	138.2^{bcd}	4.9^{b}
Beauregard	Louisiana	Orange	205.5^{cd}	8.9^{bc}	32.4^{b}	148.3^{bcd}	6.7^{b}
Kumara	New Zealand	White	79.5^{defg}	0.9^{d}	25.2^{bcd}	114.1^{cd}	10.9^{b}
Bosbok	Brazil	White	37.4^{g}	0.5^{d}	2.5^{d}	50.5^{d}	1.9^{b}
Golden Sweet	California	Cream	25.9^{g}	4.6^{cd}	31.5^{b}c	81.0^{cd}	8.9^{b}
O'Henry	Louisiana	White	55.3^{efg}	0.9^{d}	6.7^{cd}	41.2^{d}	2.1^{b}

Mean values (n=5) with the same letters in a column are not significantly different at P<0.05 (Tukey's test). ChlA: chlorogenic acid; CafA: caffeic acid; diCQA: dicaffeoylquinic acid.

grown 'Golden Sweet', 'California Organic', Honduras-grown 'Bosbok' and a purple fleshed breeding line were different from the other cultivars. In these cultivars, a peak eluting immediately before chlorogenic acid had a higher peak area than chlorogenic acid peak indicting that an isomer of chlorogenic acid may be the predominant acid in these cultivars. Caffeic acid was the least abundant phenolic acid in most of the sweetpotato

cultivars and breeding lines. The highest content of chlorogenic acid (422.4 µg/g dry weight) was present in the white-fleshed Jamaican cultivar. However, a purple fleshed breeding line had the highest amount of 3,5-dicaffeoylquinic acid (485.6 µg/g dry weight); 4,5-dicaffeoylquinic acid (284.4 µg/g dry weight); 3,4-dicaffeoylquinic acid (125.6 µg/g dry weight), and caffeic acid (20.5 µg/g dry weight) . Among all orange-fleshed cultivars and breeding lines, the cultivar 'Diane' had the highest content of individual phenolic acids. Consistent with our results, significant differences were found in chlorogenic and dicaffeoylquinic acid content of other sweetpotato cultivars and breeding lines previously analyzed (Son et al., 1991; Walter and Purcell, 1979). The high caffeic acid content in sweetpotato cultivars was associated with a defense mechanism against certain pathogen (Harrison et al., 2003). Further research is needed to find a correlation, if any, between major individual phenolic acids and disease resistance of specific cultivars.

CONCLUSIONS

A wide variation existed in phenolic content and antioxidant activity among sweetpotato cultivars and breeding lines. Information on phenolic acid profile and antioxidant activity of existing sweetpotato cultivars may assist sweetpotato breeders programs to develop cultivars with superior health benefits and high nutraceutical value. The extract from cultivars with high phenolic and antioxidant contents may have the potential for use as natural antioxidants. In addition, further research on the relationship between individual phenolic acid concentration and sweetpotato disease resistance is important.

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CHAPTER 8. SUMMARY

Phenolic compounds are important secondary metabolites in sweetpotatoes and contribute to the nutraceutical value of the edible tissue. The objectives of this study were to determine the factors affecting the phenolic composition and antioxidant activity in sweetpotatoes. In addition, methodologies for quantification of total phenolics and individual phenolic acids in sweetpotatoes were optimized. Among the two most commonly used procedures for total phenolic assay, Folin-Denis reagent provided more reliable and accurate results than Folin-Ciocalteu reagent. The best separation of individual phenolic acids with a short analysis time (< 10 minutes) was based on reversed-phase HPLC using a 5-µm, 4.6 × 250 mm column with a mobile phase consisting of 1% (v/v) formic acid aqueous solution: acetonitrile: 2-propanol (70:22:8), pH 2.5. Methanol and ethanol provided higher phenolic acid extraction efficiency than acetone, while use of hexane to remove polar lipids provided no advantage. The antioxidant activity was measured using the 1,1-diphenyl-2-picrylhydazyl (DPPH) free radical assay.

Many factors influence the concentration of total phenolics, individual phenolic acids, and antioxidant activity in sweetpotatoes. These factors include growth stage of the tissue, type of tissue, genotype, and postharvest care (curing, storage temperature, duration, and processing). Small roots at the initial stages of growth and young immature leaves contained the highest amounts of phenolic content and antioxidant activity. Sweetpotato leaf tissue had a significantly higher phenolic content and antioxidant activity than root tissue. Due to their high phenolic content and antioxidant activity, small

immature roots and leaves may be useful plant tissue sources of potential nutraceutical compounds.

Microwaving, conventional oven baking, and boiling are the most widely used methods of sweetpotato processing at home and in food-service establishments. Conventional oven baking of sweetpotatoes resulted in a higher loss of total phenolics, individual phenolic acids, and antioxidant activity compared to microwaving and boiling. Among root tissue locations, the outer periderm or skin tissue had the highest phenolic content and antioxidant potential. Functional food manufacturers interested in using sweetpotato roots as a concentrated source of phenolic compounds and antioxidants should focus on utilization of the skin tissue.

Short durations of 4 week storage at low temperature (5 °C) significantly increased phenolic compound concentration and antioxidant activity in sweetpotatoes. The concentration of phenolic compounds and antioxidant activity increased significantly when low temperature stored roots were transferred to ambient temperature (~ 22 °C). Non-cured roots accumulated a higher phenolic content and antioxidant activity than cured roots. A brief period (~3 weeks) of low temperature storage may significantly increase phenolic content and antioxidant activity without causing a loss in root marketability.

Although sweetpotatoes are well known as a nutritionally rich crop, their use in the fresh-cut industry is limited. Minimally processing of sweetpotatoes (shredding, French-fry cut, and slicing) followed by 8 days storage at 5 °C resulted in a significant increase in phenolic compounds. No tissue browning was observed after 8 days of storage and the fresh-cut product was considered to be marketable. Another factor affecting the

phenolic content and antioxidant activity was the genotype or cultivar. A purple-fleshed breeding line had the highest concentration of phenolics and antioxidant activity compared to orange- and white-fleshed cultivars.

In general, chlorogenic acid and 3, 5-dicaffeoylquinic acids were the predominant individual phenolic acids in sweetpotatoes while caffeic acid was the least abundant. Other identified phenolic acids included 4,5-dicaffeoylquinic and 3,4-dicaffeoylquinic acids. Several unidentified peaks occurring in the HPLC separation were speculated to be isomers of chlorogenic acid and 3,4,5-tricaffeoylquinic acid. Results from this research indicated the trend of changes in individual phenolic acids generally paralleled that of total phenolic content.

The understanding of the mechanisms involved in phenolic acid synthesis in sweetpotato may facilitate the use of appropriate pre- and/or postharvest treatments to enhance the nutraceutical value of sweetpotatoes. The mechanism of loss in phenolic compounds during high temperature processing requires further investigation.

VITA

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