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Phytochemical changes in phenolics, anthocyanins, ascorbic acid, and carotenoids associated with sweetpotato storage and impacts on bioactive properties

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ABSTRACT

Sweetpotato phytochemical content was evaluated in four genotypes (NCPUR06-020, Covington, Yellow Covington, and NC07-847) at harvest and after curing/storage for 4 or 8 months. Curing and storage for up to 8 months did not significantly affect total phenolic content in Covington, Yellow Covington, and NC07-847, however for NCPUR06-020, a purple-fleshed selection, total phenolic content declined mainly due to anthocyanin degradation during storage. Covington had the highest carotenoid content at harvest time (281.9 μg/g DM), followed by NC07-847 (26.2 μg/g DM), and after 8 months, total carotenoids had increased by 25% and 50%, respectively. Antioxidant activity gradually declined during storage, and freshly harvested sweetpotatoes also demonstrated higher anti-inflammatory capacity as gauged by inhibition of lipopolysaccharide-induced reactive oxygen species (ROS) in SH-SY5Y cells. Gradual changes in sweetpotato phytochemical content and antioxidant and anti-inflammatory capacity were noted during normal long-term storage, but the specific effects were genotype-dependent.

1. Introduction

Epidemiological studies have provided convincing evidence of the beneficial role of fruits and vegetables in the diet for maintenance of health and prevention of chronic diseases. This information has led to significantly increased awareness among consumers, academia, and the medical community regarding the health benefits associated with a diet rich in fruits and vegetables. Antioxidants derived from plant sources may play an important role in reducing the risk of degenerative diseases such as cancer, obesity, cardiovascular disease and diabetes (Prakash & Gupta, 2009). Bioactive secondary plant metabolites, e.g. flavonoids, carotenoids, and plant sterols, have been linked to health protection in in vitro, in vivo, and clinical research (Eastwood, 1999).

Worldwide, sweetpotato (*Ipomea batatas*) is the sixth most important food crop after rice, wheat, potatoes, maize, and cassava. More than 105 million metric tons are produced globally each year; 95% of which are grown in developing countries (CGIAR, 2010). North Carolina (NC) is the number one producer of sweetpotato in the U.S. Today more than 40% of the national supply comes from NC (NCDA & CS, 2012). The sweetpotato has been reported to have numerous health benefits including antimutagenic, antioxidant, hepato-protective, cardio-protective, and antidiabetic effects, which have been attributed to the sweetpotato's phytochemical constituents (Bovelle-Benjamin, 2007). Depending on flesh colour, sweetpotato contains a host of plant bioactive compounds, including carotenoids, anthocyanins, phenolic acids, other flavonoids and vitamin C (Truong, McFeeters, Thompson, Dean, & Shofran, 2007). It has long been known that the orange-fleshed sweetpotato contains beta-carotene, responsible for conferring pro-vitamin A activity that contributes to the prevention of vitamin A deficiencies and night blindness (van Jaarsveld et al., 2005). In addition to carotenoids, sweetpotatoes contain a unique blend of phenolic compounds such as hydroxycinnamic acids, which represent the main phenolic antioxidants in most commercially available sweetpotato cultivars (Padda & Picha, 2008). Antioxidant and free radical scavenging ability in the sweetpotato are largely attributed to phenolic content (Rabah, Hou, Komine, & Fuji, 2004). Suda et al., (2003) reviewed the accumulated research evidence supporting

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cardio-protective, hepato-protective, antidiabetic, antimutagenic properties and other physiological functions of acylated anthocyanins found in purple-fleshed sweetpotatoes.

The phytochemical content of produce can be affected by postharvest and handling procedures such as temperature, curing time, irradiation time, and exposure to light due to tissue biochemical responses. Although many fruits and vegetables are highly perishable, the time from harvest to consumption for sweetpotato can range from days or weeks to months. In temperate growing regions like the United States, roots are usually cured by holding in a properly ventilated facility maintained at about 29 °C with 85–90% relative humidity for 4–7 days (Edmunds et al., 2008). The effects of curing and storage on sweetpotatoes have been previously investigated, but earlier research focused mainly on carotenoids and vitamin C (Ezell & Wilcox, 1948, 1952), with only cursory attention to hydrophilic antioxidants, like chlorogenic acid, isochlorogenic acids, and anthocyanins. Ishiguro, Yahara, and Yoshimoto (2007) noted the lack of documented information regarding postharvest handling effects on sweetpotato composition, and also reported that the polyphenols and radical scavenging ability increased in sweetpotatoes during 37 days of cold storage. The influence of age, farming site and boiling on carotenoid content of sweetpotato has been reported, but limited information is available on the effect of storage on the phytochemical composition (K'osambo, Carey, Misra, Wilkes & Hagenimana, 2009).

The aim of this study was to elucidate the changes in the profile and content of phenolic acids, carotenoids, vitamin C and anthocyanins during the postharvest practices of curing and storage of four distinct sweetpotato genotypes grown in North Carolina. In addition, the antioxidant and anti-inflammatory potential for sweetpotatoes after long term storage was assessed.

2. Materials and methods

2.1. Chemicals and reagents

Folin-Denis reagent, sodium carbonate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox (6-hydroxy-2,5,7,8-tetramethyl chromane-2-carboxylic acid), *m*-phosphoric, *O*-phosphoric, L-ascorbic, acetic, formic, trifluoroacetic and sulphuric acids, B-carotene, dithiothreitol (DTT), butylatedhydroxytoluene (BHT), 2′,7′-dichlorofluorescin diacetate (DCF-DA), L-glutathione (GHS), penicillin–streptomycin, lipopolysaccharide (LPS), phosphate buffered saline (PBS), dimethyl sulfoxide (DMSO) and trypsin–EDTA, were purchased from Sigma Inc. (St. Louis, MO., U.S.A.). SH-SY5Y cells, DMEM, and fetal bovine serum were purchased from ATCC (Manassas, VA., U.S.A.). Cyanidin-3-*O*-glucoside, caffeic, and chlorogenic acids were purchased from Chromadex (Irvine, CA., U.S.A.). 3,4-, 3,5- and 4,5 dicaffeoylquinic acid (DCQA) were purchased from Biopurify Phytochemicals Ltd. (Sichuan, China).

2.2. Plant materials

Four sweetpotato genotypes (one commercial cultivar; Covington, and three non-commercial field trial selections; a mutant sport of Covington referred to as "Yellow" Covington, and two advanced breeding lines NC07-847, and NCPUR06-020, developed by the NCSU sweetpotato breeding program and grown at the NC Department of Agriculture and Consumer Services Horticultural Crops Research Station in Clinton, NC), were analysed in this study. Sweetpotato roots harvested in November 2010 were directly analysed without curing. Samples for storage treatments were cured at 29 °C and 85% relative humidity for 7 days, then subsequently stored in the dark at 15 °C and 80–85% humidity. Samples were taken for analysis after 4 and 8 months of storage. Before analysis, freshly harvested and stored sweetpotato samples (4 roots from each genotype) were washed, towel dried, peeled, cubed (2 cm), weighed and immediately frozen at −80 °C before lyophilization (Labconco Freezone 12, Kansas City, MO, U.S.A.). The freeze-dried material was weighed and the dry matter content was estimated by difference in weight. Lyophilized sweetpotato samples were subsequently ground into a fine powder using a fast speed grinder (IKA, Wilmington, NC, U.S.A.) and stored at −80 °C until extraction. All analyses were conducted in triplicate.

2.3. Extraction and analysis of phenolics

Extracts were prepared from lyophilized sweetpotato powder following a method described by Padda and Picha (2008), with some modifications. Briefly, 1.25 g of powdered sweetpotato was weighed into 15-mL centrifuge vials. Eight mL of 80% methanol:20% H_2O (1% acetic acid) was added and the mixture was sonicated for 10 min at room temperature. The mixture was then centrifuged at 5000 rpm for 10 min (Sorvall RC-6 plus, Asheville, NC), and the resulting supernatant was collected into a 25 mL volumetric flask. The extraction of the pellet was repeated two more times and the combined extracts were brought to a final volume of 25 mL with the extraction solvent. This solution was filtered, using a 0.20 μm PTFE syringe filter (Fisher Scientific, Pittsburg, PA), before analysis for phenolic measurements, HPLC, antioxidant activity by the DPPH assay, and anti-inflammatory & antioxidant capacity using the ROS cell bioassay, as described below.

Total phenolics were quantified by the Folin-Denis spectrophotometric assay as previously described (Singleton, Orthofer, & Lamuela-Raventos, 1999). Briefly, 0.5 mL of filtered phenolic extract, standard or blank was mixed with 8 mL of distilled water followed by the addition of 0.5 mL of Folin-Denis reagent and 1 mL of 1 N Na_2CO_3. The mixture was shaken and kept in the dark for 2 hours before being measured spectrophotometrically at 750 nm. The absorbance was measured using a Spectramax M3 plate reader with SoftMax Pro software (Molecular Devices Molecular Devices, LLC, Sunnyvale, CA). Results were expressed as chlorogenic acid equivalents per gram of dry tissue. The linear range of chlorogenic acid standard was found to be between 50 and 750 mg/mL and a calibration curve was used for quantification.

Individual phenolic acids were measured by HPLC analysis as described by Truong et al. (2007) with minor modifications. In brief, separation was achieved with an Agilent 1200 HPLC (Agilent Technologies, Santa Clara, CA) with a photodiode array (DAD) detector, and an autosampler with Chemstation software. The HPLC was outfitted with a reversed phase Phenomenex Synergi 4 μm hydro-RP 80 Å column (250 mm × 4.6 mm × 5 μm, Torrance, CA.) equipped with a guard column (Phenomenex security guard cartridge, AQC 18.4 × 3.00 mm). Samples were filtered through 0.2 μm PTFE filters (Fisher Scientific, Pittsburg, PA) before injection. The mobile phase consisted of 0.1% formic acid in H_2O (A) and 100% methanol (B). The operating conditions were: autosampler tray at 10 °C; column oven at 35 °C; constant elution flow rate of 1 mL/min. Separation of phenolic compounds started with a linear gradient of 20% B (0–10 min), 40%B (10–25 min), 90% B (25–30 min), and a post-run with 20% B, to equilibrate the column for the next injection. UV maximum absorption was recorded at 254 nm and 326 nm. Identification of phenolic compounds was based on retention time and UV spectra with reference to commercially available standards and in comparison to published data (Truong et al., 2007). Three concentrations of each phenolic acid (chlorogenic, caffeic, 3,5-, 3,4- and 4,5-DCQA) were prepared at 0.5, 0.25, and 0.125 mg/mL where 10 μL were injected as an external standard. Quantification of phenolic acids was performed using the peak areas recorded at 326 nm to generate the calibration curve for each standard. Results were expressed as milligrams per gram of dry weight tissue (mg/g DM).

Compositional analysis for anthocyanins was performed using 1200 HPLC system (Agilent Technologies Inc., Santa Clara, CA). Separation was conducted using a reversed-phase Supelcosil-LC-18 column, 250 mm × 4.6 mm × 5 μm (Supelco, Bellefonte, PA). The mobile phase consisted of 5% formic acid in H_2O (A) and 100% methanol (B). The flow rate was constant at 1 mL/min with a step gradient of 10%, 15%, 20%, 25%, 30%, 60%, 10%, and 10% of solvent B at 0, 5, 15, 20, 25, 45, 47, and 60 min, respectively. Samples were filtered through 0.2 μm nylon filters before injecting 10 μL into the HPLC column with a constant column temperature of 30 °C. Three concentrations of cyanidin-3-*O*-glucoside were prepared at 5.0, 0.25, and 0.125 mg/mL where 5 μL was injected as an external standard. Quantification of anthocyanins was performed using the peak areas recorded at 520 nm to construct the calibration curve for cyanidin-3-*O*-glucoside.

Electrospray ionisation ion-trap time-of-flight mass spectrometry (Shimadzu Scientific Instruments, Columbia, MD) was used for anthocyanin identification, formula determination and structural elucidation. This LCMS-IT-TOF system equipped with a Prominence HPLC system (SIL-20A HT autosampler, LC-20AD pump system, SDP-M20A diode array detector), is a benchtop tandem mass spectrometer designed to perform high-precision LC-MS^n analyses. HPLC was performed on the integrated LC system and the separation was performed using Shim-pack XR-ODS column (3 mm i.d. × 75 mm, 2.2 μm; Shimadzu Scientific Instruments, Columbia, MD) at 40 °C with a binary solvent system comprised of 0.1% formic acid in water (A), and methanol (B). Compounds were eluted into the ion source at a flow rate of 0.35 mL/min with a step gradient of B of 5–8% (0–5 min), 8–14% (10 min), 14% (15 min), 20% (25 min), 25% (85 min), 5% (35 min) and 5% (40 min). Ionisation was performed using an ESI source in the positive and negative modes. Compounds were characterised and identified by their MS, MS/MS spectra and LC retention times and by comparison with available reference samples.

Total monomeric anthocyanins were also quantified with the pH differential assay as described by Lee, Durst, and Wrolstad (2005). Concentrations were expressed as mg cyanidin 3-*O*-glucoside equivalents per gram of dried sample.

2.4. Extraction and analysis of ascorbic acid

Ascorbic acid was extracted as described by Hernandez, Lobo, & Gonzalez 2006) with some modifications. Freeze-dried sweetpotato samples (0.5 g) were extracted with 8 mL extraction solvent (3% meta-phosphoric acid and 8% acetic acid in water). The resulting mixture was sonicated for 5 min and centrifuged at 4000 rpm for 20 min at 4 °C. This procedure was repeated two times and the resulting supernatants were collected and brought to final volume of 25 mL. Immediately, 800 μL of the filtered extract (0.2 μm cellulose syringe filter) was added to 200 μL of DTT (20 g/L) as a reducing agent, left for 2 h before HPLC analysis. Precautions were made to avoid light exposure throughout the whole procedure. HPLC separation was achieved using an Agilent 1200 HPLC equipped with Phenomenex Synergi 4 μm hydro-RP 80A column (250 mm × 4.6 mm × 5 μm, Torrance, CA) where 20 μL was injected for each sample. Isocratic elution was performed with a mobile phase of Millipore water acidified to pH 2.2 with sulphuric acid at a constant flow rate of 1.2 mL/min. Total content of ascorbates was performed from the peak areas recorded at 245 nm with reference to the calibration curve obtained with ascorbic acid reference.

2.5. Extraction and analysis of carotenoids

Extraction and chromatographic separation of carotenoids was performed according to the procedure described before (Kurilich & Juvik, 1999) with modifications. Briefly, powdered sweetpotato samples were placed in 50 mL tubes and 9 mL of ethanol/BHT (0.001%) were added. Appropriate sample weights (based on carotenoid concentration) of 0.1 g, 0.4 g, 0.8 g, and 1.0 g for Covington, NC07-847, Yellow Covington, and NCPUR06-020, respectively were used. Samples were placed in warm water bath (40 °C) for 10 min with occasional vortexing. Samples were then placed on ice and 3 mL of cold water was added followed by 3 mL hexane/BHT (0.001%) and centrifuged at 3000 rpm for 10 min at 10 °C. A glass rod was used to break up clumps if any to complete extraction. A glass pipette was used to transfer the supernatant into a new test tube placed on ice. The hexane extraction was repeated two more times to collect approximately a total of 9.0 mL. Hexane was removed using a Buchi Syncore evaporator equipped with a controlled temperature shaker (30 °C) and a vacuum pump (Buchi Corp., Postfach, Switzerland) where a dry residue was obtained. Precautions were taken to avoid oxidation by air and direct light, where all procedures were carried out under gold/yellow light. Sample residues were re-suspended in 1 mL hexane/BHT (0.001%) extraction solution, filtered using 0.2 μm PTFE syringe filters (Fisher Scientific, Fair Lawn, NJ) into 2 mL HPLC amber vials where 5 uL were immediately injected for HPLC analysis.

HPLC analysis was performed using an Agilent 1260 Infinity system (Agilent Technology Inc., Santa Clara, CA) equipped with diode array detector and autosampler (4 °C). Carotenoids were separated and analysed using a reversed-phase C-30 column 250 × 4.6 mm and 5 μm particle size (YMC America, Inc. Allentown, PA). Solvent A was 0.05% ammonium acetate in water and B was acetonitrile, methanol, dichloromethane, BHT at a ratio of 50:13:33:4 v/v with 0.1% of triethylamine (TEA) was added. The solvent gradient system was performed as 95%, 95%, 100%, 100%, 95%, and 95% of B at 0, 10, 20, 50, 55, and 60 min, respectively. Flow rate was constant at 1.8 mL/min. and column temperature was 35 °C. Carotenoids were detected at 450 nm. All samples were analysed in triplicate. Samples and standards were injected at 5 μL. A fourth sample for each genotype was spiked with a standard reference by adding 15 μg beta-carotene at beginning of extraction. This was to confirm the identification of the compound and estimate the extraction recovery for carotenoids in the sweetpotato tissues. Carotenoids were monitored at UV maximum absorption of 450 nm and DAD spectral data from 250 to 550 nm were stored to examine spectrum peaks for carotenoids. Since the beta-carotene was the major carotenoid in sweetpotato tissue and available as commercial standard, it was used for quantification for all carotenoids including minor carotenoids detected in the samples. Minor carotenoids were pooled together and designated as other carotenoids. Carotenoid concentrations were calculated using the beta-carotene standard curve of 0.125, 0.063, 0.031, 0.016, and 0.008 mg/mL and data were presented in μg/g dry weight.

2.6. DPPH radical scavenging activity

The DPPH radical scavenging activity assay was performed on the phenolic extracts, prepared as described above, following the procedure described by Truong et al. (2007). Samples from the NCPUR06-020 sweetpotato were diluted 10 times with 80% methanol, while the three other genotype extracts were used undiluted. An aliquot (100 μL) of each sample was pipetted into 3.9 mL of DPPH solution (0.08 M in 95% ethanol) to initiate the reaction. After a reaction time of 3 h at ambient temperature the reaction had reached completion (Teow et al., 2007). The decrease in absorbance of DPPH free radicals was read at 515 nm against ethanol as a blank using a Shimadzu UV-2450 spectrophotometer. Trolox (0, 100, 200, 300, 400, and 500 μM) was used as a standard antioxidant compound. Analysis was performed in triplicate for each sample and each concentration of standard. The antioxidant activity

was reported in μmol of Trolox equivalents per gram dry weight tissue (μmol TE/g DM).

2.7. Quantification of reactive oxygen species production in SH-SY5Y cells

Anti-inflammatory capacity of sweetpotato was further assessed using a standard LPS-induced reactive oxygen species (ROS) accumulation neuronal model for inflammation (Gustafson, Yousef, Grusak, & Lila, 2012). Briefly, SH-SY5Y human neuroblastoma cells were cultured in a humidified atmosphere (5% CO_2, 37 °C) and grown in DMEM:F-12 medium supplemented with 10% fetal bovine serum, 100 U/mL Penicillin, and 100 U/mL Streptomycin in 100 mm dishes. Cultures were replenished with fresh medium every 48–72 h. Cells were harvested with trypsin (0.5 mg/mL)/EDTA (0.2 mg/mL) in PBS, centrifuged for 2 min at 700*g*, suspended in new medium, and plated to tissue culture-treated six well plates for experimentation. Cell cultures were maintained for 72 h and then serum starved overnight prior to treatment. For this set of experiments, stock solutions were prepared by drying the sweetpotato extracts and then suspending the dried material in 0.5 mL DMSO. Stock solutions of extracts were diluted 1:20 immediately prior to use in 1x PBS and then further diluted in DMEM at a final assay concentration of 100 μg/mL. Cultures were incubated (1 h) with 1 mM GSH or 100 μg/mL sweetpotato extract in conjunction with DCF-DA (20 μM). Cultures were then exposed to 1 μg/mL LPS for 30 min – during which DCF-DA is oxidised to DCF by H_2O_2. Cells were washed and harvested in PBS and 200 μL of sample was transferred to a black 96 well Corning plates (Fisher Scientific, Pittsburg, PA) for analysis. ROS formation was quantified as maximum DCF-Fluorescence intensity using a Spectra Max M3 multi-mode microplate reader (Sunnyvale, CA) (485 nm excitation, 538 emission). All values were normalised to the control cells that were only exposed to DCF-DA.

2.8. Statistical analysis

A completely randomized design (CRD) was used for data analysis of variance (ANOVA) using SAS Statistical Analysis System (v. 5.1, SAS Institute Inc., Cary, NC, U.S.A.). PROC GLM procedure was used to detect differences among genotypes and storage treatments at $p < 0.05$ using protected LSD test. A total of four genotypes (Covington, Yellow Covington, NC07-847, and NCPUR06-020) were included in the analysis where each genotype had three storage treatments (freshly harvested, cured and stored for 4 months, and cured and stored for 8 months). The results are expressed as mean concentrations of the three samples extracted and analysed per treatment ($n = 3$) for all variables. Variables included total phenolics, total anthocyanins, chlorogenic acid, caffeic acid, 3,4-, 3,5- and 4,5-dicaffeoylquinic acid (DCQA), vitamin C, B-carotene, other carotenoids, total carotenoids and DPPH. Pearson's correlation coefficients among all variables evaluated in this study were also computed.

3. Results and discussion

3.1. Dry matter content

Sweetpotato genotypes used in this study ranged in dry matter content (DM) from 19.7% for Covington to 33.7% for NCPUR06-020 (for freshly harvested roots). After 8 months storage, the degree of dry matter change was genotype-dependent. There was a decline in DM% at 8 months storage for NCPUR06-020 and NC07-847, while there was a slight increase in DM for Covington and no significant change for Yellow Covington (Table 1). Biochemical changes during storage were studied by Zhang, Wheatley, & Corke (2002), where they found a slight decrease in DM over 180 days of storage depending on genotype. The decrease in DM was correlated with the decrease in starch content and an increase in α-amylase activity in the first 60 days of storage, where α-amylase activity varied among genotypes (0.4–1.44 Ceralpha unit/g).

3.2. Total phenolics, anthocyanins, phenolic acids, and ascorbic acid (vitamin C)

Total phenolics (TP) were measured in the four sweetpotato genotypes at harvest time, and after curing for 4 and 8 months of storage. Results were expressed as mg/g chlorogenic acid equivalent based on dry weight and presented in Table 1. The purple-fleshed genotype, NCPUR06-020, contained over 13-fold higher TP compared to the other three (orange/yellow-fleshed) genotypes. Total phenolics in NCPUR06-020 were 39.92 mg/g at harvest time, as compared to an average of 2.83 mg/g for the other genotypes. With NCPUR06-020, there was no significant change in TP concentration at 4 months of storage, however, at 8 months, the TP content dropped to 34.19 mg/g, which represented a reduction of about 14.4% compared to the freshly harvested roots. There was a slight decline with no significant

Table 1
Total phenolics, total anthocyanins, phenolic acids, ascorbic acid (mg/g DM), and DPPH antioxidant activity (μM Trolox/g DM) estimated in freshly harvested sweet potatoes, and after curing/storage for four and eight months post-harvest.

Genotype (flesh colour)	Storage time	Dry matter (%)	TP[1]	ANC[2]	Phenolic acids						Ascorbic acid[3]	DPPH μM Trolox
					ChA[3]	CA[3]	4,5-DCQA[3]	3,5-DCQA[3]	3,4 DCQA[3]	Total		
NCPUR06-020 (purple)	Fresh	33.70^{a}	39.92^{a}	8.49^{a}	10.76^{a}	0.12^{c}	1.94^{a}	3.78^{c}	0.16^{c}	16.76 ^{a}	0.66^{d}	8.47^{a}
	4 months	32.75^{ab}	40.08^{a}	7.12^{b}	5.39^{b}	0.16^{b}	1.25^{b}	4.82^{a}	0.32^{b}	11.94^{b}	0.68^{d}	7.87^{b}
	8 months	30.77^{b}	34.19^{b}	4.96^{c}	3.00^{c}	0.19^{a}	1.01^{c}	4.54^{b}	1.20^{a}	9.94^{c}	0.23^{g}	7.43^{c}
Covington (orange)	Fresh	19.69^{h}	2.87^{c}	nd	0.49^{f}	0.05^{hi}	0.02^{ef}	0.32^{e}	0.02^{ef}	0.90^{d}	0.87^{a}	0.51^{d}
	4 months	19.10^{h}	2.49^{c}	nd	0.31^{g}	0.07^{f}	0.03^{e}	0.13^{g}	0.01^{fgh}	0.55^{e}	0.57^{e}	0.37^{de}
	8 months	22.7d^{ef}	2.48^{c}	nd	0.20^{h}	0.05^{h}	0.01^{f}	0.07^{h}	0.01^{gh}	0.34^{f}	0.49^{f}	0.35^{de}
Yellow Covington (light yellow)	Fresh	20.12^{hg}	2.78^{c}	nd	0.54^{e}	0.05^{hi}	0.02^{ef}	0.34^{e}	0.02^{efg}	0.97^{d}	0.77^{b}	0.53^{d}
	4 months	21.12^{fgh}	2.65^{c}	nd	0.56^{de}	0.05^{h}	0.06^{d}	0.26^{f}	0.02^{d}	0.95^{d}	0.49^{f}	0.50^{d}
	8 months	22.47^{efg}	2.32^{c}	nd	0.15^{i}	0.05^{i}	0.03^{e}	0.10^{h}	0.02^{e}	0.35^{f}	0.12^{i}	0.28^{e}
NC07-847 (yellow)	Fresh	27.53^{c}	2.83^{c}	nd	0.59^{d}	0.06^{g}	0.01^{f}	0.40^{d}	0.01^{h}	1.07^{d}	0.74^{c}	0.56^{d}
	4 months	24.82^{de}	2.55^{c}	nd	0.23^{h}	0.08^{d}	0.05^{d}	0.13^{g}	0.02^{ef}	0.51^{e}	0.47^{f}	0.43^{de}
	8 months	25.07^{d}	2.52^{c}	nd	0.10^{j}	0.07^{e}	0.05^{d}	0.13^{g}	0.03^{d}	0.38^{f}	0.19^{h}	0.35^{de}

TP: total phenolics; ANC: anthocyanins; ChA: chlorogenic acid; CA: caffeic acid; DCQA: dicaffeoylquinic acid; nd: not detected; 1: quantified by Folin Denis assay as chlorogenic acid equivalent; 2: quantified by HPLC as cyanidin-3-glucoside equivalent; 3: quantified by HPLC using standard references. Means with different letters within the same column are significantly different at $p < 0.05$.

differences in the total phenolic content in Covington, Yellow Covington, and NC07-847 after 4 or 8 months of storage. Anthocyanin content of NCPUR06-020 genotype (purple flesh), as measured by HPLC (Table 1), declined with storage, from 8.49 mg/g for freshly harvested, 7.12 and 4.96 mg/g after 4 and 8 months of storage, respectively. Purple sweetpotao is known to contain acylated anthocyanins that may not respond to pH changes the same way as non-acylated anthocyanins (Lee et al., 2005) but our results from pH differential assay (6.57, 6.38 and 5.17 mg/g for fresh roots, and after 4 and 8 months, respectively) were well correlated with the HPLC quantitative analysis. The decline of ANC content correlates with the decrease of total phenolic content of this genotype with storage (r^2 = 0.91). Anthocyanins can be degraded by enzyme systems in plant tissues such as glycosidases (anthocyanases), polyphenoloxidases (PPO) and peroxidases (Shi, Bassa, Gabriel, & Francis, 1992). Anthocyanin components detected in purple sweetpotato were mainly cyanidin and peonidin glycosides acylated with caffeic acid, ferulic acid and p-hydroxybenzoic acids, in agreement with previous reports (Suda et al., 2003). HPLC (Fig. 1), MS and MS/MS analyses for anthocyanins enabled us to track the changes in profile and concentration in NCPUR06-020 over the duration of storage. Four major anthocyanins were identified including cyandin-3-caffeoyl-*p*-hydroxybenzoylsophoroside-5-glucoside (YGM-1a) (**1**), cyanidin 3-caffeoylsophoroside-5-glucoside (YGM-2) (**2**), cyanidin 3-(6″-caffeoyl-6‴-feruloylsophoroside)-5-glucoside (YGM-3) (**3**), and peonidin 3-caffeoyl-*p*-hydroxybenzoyl-sophoroside-5-glucoside (YGM-5a) (**4**). There was a noticeable decrease in anthocyanins **1** and **3** at 4–8 months of storage. Anthocyanin **2** increased at 4 months, and then exhibited a sharp drop at 8 months of storage. Levels of the least abundant anthocyanin identified, **4**, declined slightly at 4 months, then increased after 8 months as shown in Fig. 1.

HPLC analysis for individual phenolic acids indicated that chlorogenic acid was the major phenolic compound in all investigated sweetpotato genotypes. NCPUR06-020 contained 10.76 mg/g (DM), which represented about 20-fold the concentration in the orange/yellow-fleshed sweetpotato genotypes (0.49, 0.45 and 0.59 mg/g for Covington, Yellow Covington and NC07-847, respectively). There was a significant decrease in chlorogenic acid concentration (59–83%) in all sweetpotato genotypes at 8 months of storage (Table 1). Caffeic acid as well as the three dicaffeoylquinoic acids (3,5-, 3,4- and 4,5-DCQA) were also highly accumulated in NCPUR06-020, showing an average of 10-fold the concentrations present in the other investigated genotypes. Not all phenolic acids behaved similarly to chlorogenic acid upon storage; while each of caffeic acid, 3,5- and 3,4-DCQA significantly increased in NCPUR06-020 (1.5, 1.2 and 7.5-fold, respectively), 4,5-DCQA concentration dropped (52%) at 8 months of storage. The NC07-847 genotype exhibited a significant increase in caffeic acid, 4,5- and 3,4-DCQA, with a decrease in 3,5-DCQA (Table 1). However, phenolic acid patterns of change were not parallel for the other two genotypes (Covington and Yellow Covington), indicating genotype-dependent changes in phenolic acid content during storage.

The decrease in phenolic component concentration during storage can be attributed to the degradation of polyphenols through storage conditions (15 °C, 85% RH) by an active enzyme system in the tissue including anthocyanases, polyphenol oxidase and peroxidase (Shi et al., 1992). The increase in caffeic acid content in NCPUR06-020 during storage can be attributed to accumulation of the degradation products of chlorogenic acid, isochlorogenic acid and acylated anthocyanins present in this genotype (Shi et al., 1992). On the other hand, a report by Padda and Picha (2008) indicated an increase in total phenolic content in cured and non-cured sweetpotato roots after 2 weeks at low storage temperature (5 °C). Hasegawa, Johnson, and Gould (1966) compared the chlorogenic acid content of potato tubers when stored at 0, 4.4 and 15.5 °C. Their results indicated that storage at 15.5 °C did not affect the chlorogenic acid concentration, but accumulation of chlorogenic acid increased only at low temperatures. They attributed the increase of chlorogenic acid during cold storage to the accumulation of sugars, which act as substrates for the synthesis of this compound. Ishiguro et al. (2007) reported that chlorogenic acid concentration increased significantly in sweetpotato stored at 5 °C compared to 15 °C, after 37 days.

Ascorbic acid (vitamin C) concentration was highest in Covington (0.87 mg/g DM) followed by Yellow Covington, NC07-847, and NCPUR06-020 (0.77, 0.74, and 0.66 mg/g, respectively). Ascorbic acid contents observed in this study were in the range of previously reported values by Huang, Chang, and Shao (2006). Storage caused a gradual decrease in ascorbate concentration in all genotypes (Table 1). Reddy and Sistrunk, (1980) indicated a 25% decrease in ascorbic acids after 7 months of storage. Other investigators have demonstrated a decrease in ascorbic acid during storage of sweetpotato (Ezell and Wilcox, 1952).

3.3. Carotenoids

Carotenoids were analysed by HPLC for beta-carotene, the major carotenoid, and other minor carotenoids quantified as B-carotene equivalents as μg/g DM. Beta-carotene eluted at 38 min,

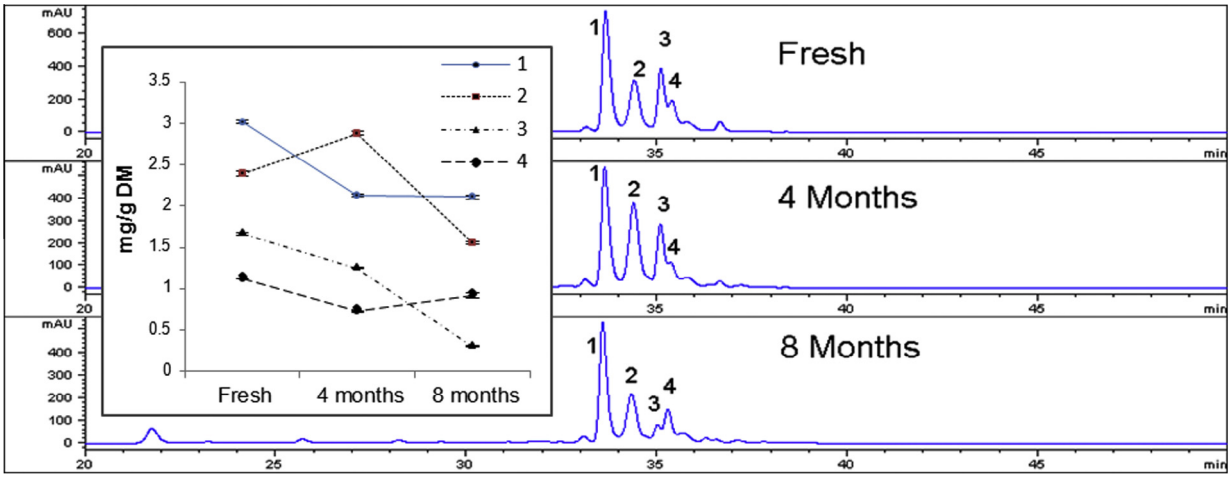

Fig. 1. HPLC chromatogram for anthocyanins in freshly harvested NCPUR06-020 sweetpotatoes (fresh) and after curing and storage (at 4 and 8 months), measured at a maximum absorption of 520 nm. **1** = cyanidin-3-caffeoyl-*p*-hydroxybenzoylsophoroside-5-glucoside (YGM-1a); **2** = cyanidin 3-caffeoylsophoroside-5-glucoside (YGM-2); **3** = cyanidin 3-(6''-caffeoyl-6'''-feruloylsophoroside)-5-glucoside (YGM-3); **4** = peonidin 3-caffeoyl-*p*-hydroxybenzoyl-sophoroside-5-glucoside (YGM-5a).

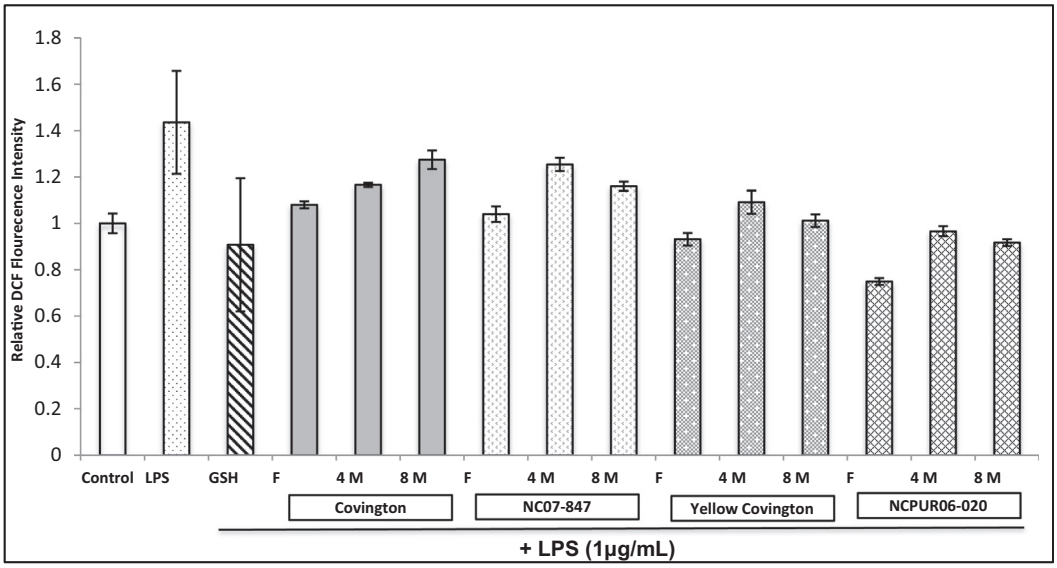

Fig. 2. The ability of sweetpotato extracts to inhibit LPS-induced accumulation of ROS in SH-SY5Y cells. Cells were incubated with GSH (1 mM) or sweetpotato extract (100 µg/mL) in DCF-DA (20 µM) prepared media, or DCF-DA media only (control), prior to LPS stimulation (30 min). All data represent the mean of at least three independent experiments ±SD. GSH: glutathione, F: freshly harvested, M: months.

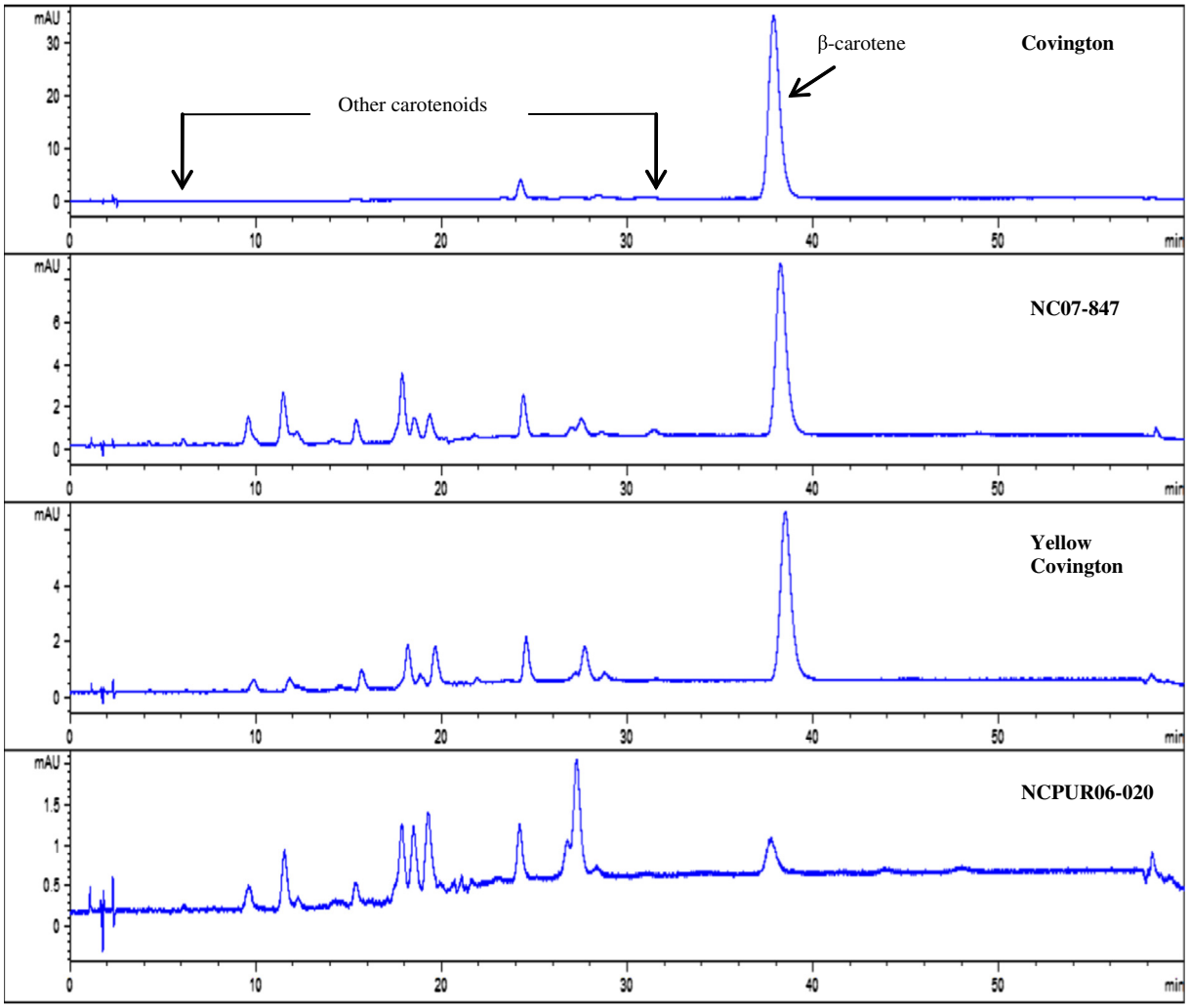

Fig. 3. Qualitative HPLC profiles for carotenoids in 4 sweetpotato genotypes stored for 8 months measured at UV maximum absorption of 450 nm. Chromatograms were set at different scales to clearly show the minor carotenoids. Sample weights for each genotype were based on relative carotenoid concentrations.

Table 2
Carotenoid content estimated in freshly harvested sweet potatoes, and after curing and storage for four and eight months post-harvest.

Genotype (flesh colour)	Storage time	Beta-carotene* μg/g DM (%)	Other carotenoids* μg/g DM	Total carotenoids μg/g DM
Covington (orange)	Fresh	253.3^{c} (89.85)	28.6^{b}	281.9^{b}
	4 months	260.1^{b} (90.88)	26.1^{c}	286.2^{b}
	8 months	291.1^{a} (87.31)	42.3^{a}	333.4^{a}
NC 07-847 (yellow)	Fresh	7.3^{fg} (27.86)	18.9^{d}	26.2^{e}
	4 months	23.3^{e} (55.34)	18.9^{d}	42.1^{d}
	8 months	32.8^{d} (62.00)	20.1^{d}	52.9^{c}
Yellow Covington (light yellow)	Fresh	9.1^{f} (53.85)	7.8^{e}	16.9^{fg}
	4 months	12.9^{f} (58.90)	9.0^{e}	21.9^{ef}
	8 months	8.1^{f} (65.85)	4.2^{f}	12.3^{g}
NCPUR06-020 (purple)	Fresh	1.0^{gh} (50)	1.0^{g}	2.0^{h}
	4 months	0.2^{h} (6.45)	2.9^{f}	3.1^{h}
	8 months	0.5^{h} (14.29)	3.0^{f}	3.5^{h}

* Carotenoids were quantified by HPLC using a commercial standard of beta-carotene. Means with different letters within each column are significantly different at $p < 0.05$.

while other carotenoids eluted from 9 to 31 min (Fig. 3). Their identities were verified based on UV absorption and the corresponding spectra stored with DAD data, and in comparison with published data (Ishiguro, Yoshinaga, Kai, Maoka, & Yoshimoto, 2010; Teow et al., 2007). Carotenoid content in sweetpotatoes largely varies depending on cultivar and growing environment; however our values appeared to be in the range of previously published data (Dincer et al., 2011). It was noted that while Covington had the highest beta-carotene ratio (approx. 90% of total), NC07-847 and Yellow Covington had about only half of their carotenoids in the form of B-carotene. Yellow Covington was originally derived from Covington as a sport which had lost one or more enzymes coding for beta-carotene. As expected, the purple fleshed clone, NCPUR06-020, had the lowest accumulation of beta-carotene; other carotenoids were observed in this clone albeit at very low levels. Our data were consistent with a previous report, which cited from 50 to 900 μg/g total carotenoids, based on fresh weight, over a large survey of sweetpotato cultivars (Hagenimana, Carey, Gichuki, Oyunga, & Imungi, 1999).

The carotenoid profile for the sweetpotato genotypes after storage for 8 months is illustrated in Fig. 3. In general, there was a significant variation of total carotenoid content among genotypes. Covington, the orange fleshed genotype, contained the highest concentrations of beta-carotene and total carotenoids (253.3 and 281.9 μg/g DM, respectively) in the freshly harvested roots (Table 2). After 4 months of curing and storage, there was a slight increase in beta-carotene with a decrease in other minor carotenoids, but there was a non-significant increase in total carotenoids. At 8 months of storage, total carotenoid content increased for Covington by approx. 18.3%. Beta-carotene concentration over the storage time increased even more dramatically (4.5-fold) for the yellow-fleshed genotype, NC07-847 (7.3, 23.2, and 32.8 μg/g in freshly harvested, 4 months, and 8 months, respectively). Total carotenoids increased approx. 50% at 8 months of storage (26.2–52.9 μg/g in fresh and 8 months storage, respectively) (Table 2). Yellow Covington and NCPUR06-020 did not show significant changes in their carotenoid content during storage; however these genotypes had low carotenoid concentration from the onset. The Covington and NC07-847 genotypes, with the highest carotenoid concentration among investigated genotypes, showed an apparent colour change (more of the orange or yellow colour) at 8 months of storage compared to the freshly harvested sweetpotato reflecting the carotenoid content in their tissues, where the colour was associated with the pigmented beta-carotene and other carotenoids. It was reported that sweetpotatoes contain, in addition to beta-carotene in all-*trans* form, isomers of *cis* beta-carotenes and other minor carotenoids such as lutein, and zeaxanthin (Hagenimana et al., 1999; Ishiguro et al., 2010; Purcell & Walter, 1968).

After curing, changes in the carotene content varied widely during storage, while some varieties showed short rapid increase, others showed gradual increase. Previously reported data on the interaction between storage and carotenoid content varies widely with either an increase (Ezell and Wilcox, 1952) or a decrease in carotenoids content with storage (Mitchell & Lease, 1941). Ezell and Wilcox (1948) indicated that genotype is the main factor in determining the pro-vitamin A values of sweetpotatoes, and that there was an absolute increase in the carotene content during curing and storage. Reddy and Sistrunk (1980) indicated that stored roots reflected more light ('*L*') value, were more orange, and contained more carotenoids (an increase of ~25%) compared to freshly harvested roots.

3.4. Antioxidant activity and inhibition of LPS-induced inflammation

DPPH radical scavenging activity was used to gauge the antioxidant activity of the sweetpotato extracts. Similar to a previous report (Teow et al., 2007), the DPPH values were highly correlated with total phenolic content ($R^2 = 0.997$). DPPH antioxidant activity ranged from 8.47 to 0.28 μM Trolox/g DM (Table 1). The high antioxidant activity of NCPUR06-020 can be attributable to the combination of high levels anthocyanin and phenolic acids in this genotype, compared to the other orange/yellow-fleshed roots.

The ability of sweetpotato phenolic extracts to inhibit LPS-induced ROS accumulation in SH-SY5Y human neuroblastoma cells is shown in Fig. 2. Freshly harvested sweetpotato extracts showed more anti-inflammatory capacity than stored samples in all genotypes tested; which correlated well with the measured DPPH radical scavenging activity. The anti-inflammatory bioactivity of NCPUR06-020 exceeded all of the other genotypes, even after 8 months of storage and this correlates well with the phenolic content and DPPH antioxidant activity for this genotype. ROS is known to enhance pro-inflammatory signalling. An increase in intracellular ROS is known to activate transcription factors such as NF-$_k$B and increase gene expression such as of PGE_2 and TNF-α associated with inflammation (Rahman, Biswas, & Kirkham, 2006). The antioxidant and potential anti-inflammatory activities are suggest significant health benefits may be associated with sweetpotato consumption, as these mechanisms are inhibitory to a host of chronic human disease conditions.

4. Conclusions

Total phenolics, total anthocyanins as well as individual phenolic acids (chlorogenic, caffeic and DCQA) were highly concentrated in the purple genotype NCPUR020-20 compared to the other three yellow/orange genotypes. Caffeic acid, a phenolic component

present at low concentrations, was more stable and showed increased over storage in two genotypes. Vitamin C (ascorbic acid) concentration varied among genotypes and decreased gradually during storage. DPPH radical scavenging activity was highly correlated with total phenolic content. Significant anti-inflammatory capacity was present in sweetpotato extracts especially from freshly harvested samples. Carotenoids were highly concentrated in the orange-fleshed Covington, followed by the yellow-fleshed genotype NC07-847. Beta-carotene was the predominant carotenoid in these two genotypes. The purple genotype, NCPUR020-020, had low carotenoid content. Unlike phenolics and vitamin C, there was an increase in carotenoid concentration in the Covington and NC07-847 genotypes after eight months of storage. Phytochemical content (TP, ANC, phenolic acids, and carotenoids) pre and post storage were genotype-dependent parameters in sweetpotatoes, as were antioxidant and anti-inflammatory properties associated with sweetpotato genotypes.

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