Cooking Methods and Storage Treatments of Potato: Effects on Carotenoids, Antioxidant Activity, and Phenolics

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Abstract The influence of genotype, cooking method, and storage treatments on potato compounds associated with improved human health was analyzed. Antioxidant activity (AA), total phenolics (TP), and total carotenoids (xanthophyll carotenoids, CAR) were determined in eight genotypes using 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, and spectrophotometric absorbance, respectively. Individual phenolic and carotenoid composition was analyzed using high performance liquid chromatography (HPLC) in three genotypes of potato. Samples were subjected to a combination of storage conditions for approximately 4 months (non-stored or stored for 110 days at either 4°C, 4°C with an additional 10 days of reconditioning at 20°C, or 20°C storage) and cooking methods (baking, boiling, frying, or microwaving); an uncooked sample was used as a control. The non-stored samples had lower amounts of CAR, AA, and TP along with the individual compounds compared to the various storage regimes, while the recondition storage treatment produced equal or higher levels of TP and individual phenolics than any other storage regime. No cooking and boiling resulted in significantly lower AA and TP, as compared to baking, frying and/or microwaving. Baking, frying and/or microwaving also increased the levels of chlorogenic acid, caffeic acid, (-) epicatechin, p-coumaric acid and vanillic acid, but decreased quercetin dihydrate when compared to uncooked samples. Most health promoting compounds were enhanced by one or both postharvest processing parameters (storage and cooking); however, t-cinnamic acid, and lutein were not affected.

Department of Horticultural Sciences, Texas A&M University, College Station, TX 77843-2133, USA e-mail: jcmillerjr@tamu.edu Resumen Se analizó la influencia del genotipo, del método de cocinado y de tratamientos en el almacén, sobre los compuestos de papa asociados con el mejoramiento de la salud humana. Se determinó la actividad antioxidante (AA), fenoles totales (TP), y carotenoides totales (carotenoides xantofilicos, CAR), en ocho genotipos, usando 2,2-difenil-1picrilhidrazilo (DPPH), el reactivo de Folin-Ciocalteau, y la absorbancia espectrofotométrica, respectivamente. Se analizaron los fenoles individuales y la composición de los carotenoides usando cromatografía de líquidos de alta resolución (HPLC) en tres genotipos de papa. Las muestras estuvieron sujetas a una combinación de condiciones de almacenamiento por aproximadamente cuatro meses (sin almacenar, o almacenadas por 110 días ya fuera a 4°C, a 4°C con diez días adicionales de reacondicionamiento a 20°C, o a 20°C en el almacén), y métodos de cocinado (horneadas, hervidas, fritas, o en el horno de microondas); se usó una muestra sin cocinar como testigo. Las muestras sin almacenar tuvieron las cantidades más bajas de CAR, AA, y TP, junto con los compuestos individuales comparados a los diversos regímenes de almacenamiento, mientras que el tratamiento de reacondicionamiento en el almacén produjo niveles iguales o mayores de TP y de fenoles individuales que cualquier otro régimen de almacenamiento. Sin cocinar y hervidas resultaron en contenido más bajo de AA y TP, comparadas con el horneado, fritas, y/o en el horno de microondas. Horneadas, fritas y en microondas también aumentaron los niveles de ácido clorogénico, ácido caféico, (-) epicatequina, ácido p-coumárico, y ácido vainillico, pero disminuyó la quercetina dihidratada cuando se les comparó con las muestras sin cocinar. Se incrementaron la mayoría de los compuestos que promueven la salud por uno o los dos parámetros de procesamiento postcosecha (almacenamiento y cocinado); no obstante, no se afectaron el ácido t-cinnamico y la luteína.

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Keywords Phytochemical · Antioxidant capacity · Thermal processing · Baking · Boiling · Frying · Microwaving

AU	 evia	

CAR	carotenoid content
TP	total phenolics
AA	antioxidant activity

Introduction

Human epidemiological and laboratory-animal studies have associated consumption of fruits and vegetables with a decreased risk of chronic disease (Ames et al. 1993). Fruits and vegetables are known to be rich in fiber, vitamins, and minerals; however, much emphasis is now placed on other compounds, referred to as phytochemicals or phytonutrients, as being responsible for the reduction of chronic disease development. These compounds are non-nutrient plant compounds with discrete bio-activity towards animal biochemistry and metabolism and are classified as carotenoids, phenolics, alkaloids, nitrogen-containing compounds, and organosulfur compounds (Dillard and German 2000; Liu 2004). The majority of phytochemical research has been conducted on phenolics and carotenoids. Phenolics have one or more aromatic rings with one or more hydroxyl groups and include the groups of phenolic acids, flavonoids, stilbenes, coumarins, and tannins. Carotenoids are characterized by a 40-carbon isoprene chain, may be cyclic at one or both ends and include the compounds alpha and beta-carotene, beta-cryptoxanthin, lutein, lycopene, and zeaxanthin (Liu 2004). Both phytochemical classes have been characterized as antioxidants, substances that prevent oxidation and protect against damage by reactive oxygen species (Halliwell et al. 1995). This mechanism amongst others has been assumed to halt the progression and proliferation of chronic illnesses including cancer, cardiovascular disease, diabetes, macular degeneration, and cataracts (Willcox et al. 2004).

Potatoes are a major contributor to dietary phytochemical concentrations due to consumption levels. In a dietary recall in the 2003–2004 National Health and Nutrition Examination Survey (NHANES), potato was the most consumed vegetable in adolescents and adults in the United States (Kimmons et al. 2009). Potato's dietary phytochemical contribution can also be attributed to its inherent level of these health-promoting compounds. The antioxidant activity of potatoes has been identified to be higher than other vegetables, including onion, carrot and bell pepper, and these values appear to be correlated with phenolic content (Al-Saikhan et al. 1995). Major phenolics include chlorogenic acid and caffeic acid; values vary by genotype and some genotypes contain more

than 500 μ g gfw⁻¹ of chlorogenic acid (Reddivari et al. 2007b). Potatoes contain significant levels of a group of carotenoids called xanthophylls, most notably lutein and zeaxanthin. Total xanthophyll concentration varies between genotypes, and white to yellow-flesh, domestic potatoes may contain up to 100 μ g 100 gfw⁻¹, while certain South American genotypes contain over 2,000 μ g 100 gfw⁻¹ (Brown 2004).

Phytochemical levels in potatoes have been previously analyzed immediately after harvest; however, domestically consumed potatoes are stored, sometimes for extended periods, and are cooked, both of which may significantly impact nutritional levels. In order to preserve quality and to satisfy demand throughout the year, potatoes are recommended to be stored at 4°C for the fresh market to 4 to 10°C for processing (Rastovski et al. 1987). These low-temperature storage conditions cause starch to break down and reducing sugar levels to increase, which produces unfavorable darkened processed products (Burton 1969). This phenomenon can be reversed partially or entirely if coldstored tubers are transferred to temperatures above 10°C prior to processing, referred to a reconditioning or curing procedure (Coffin et al. 1987; Blenkinsop et al. 2002; Isherwood 1973). Reconditioning treatments may also mimic cold-stored potatoes that sit on a home shelf for several days before cooking.

Potato phytochemicals appear to be influenced by storage conditions. Percival and Baird (2000) reported losses in chlorogenic acid, while others noted increases in carotenoid content (Bhushan and Thomas 1990; Janave and Thomas 1979), antioxidant activity (Rosenthal and Jansky 2008), and chlorogenic acid (Leja 1989) with conditions of cold-storage. Differences between study designs were noted for time of storage, temperature, humidity, light levels of storage, and differences in sample preparation.

Discrepancies between studies on the influence of domestic cooking on potato phytochemical and other nutrient levels have also been noted. In some studies, when compared to uncooked samples, cooked potatoes had measurable decreases in chlorogenic acid, phenolic content, and glycoalkaloid content (Dao and Friedman 1992; Tudela et al. 2002); other studies reported no differences in chlorogenic acid and caffeic acid (Andlauer et al. 2003); and some studies reported increases in phenolic acids, antioxidants, and glycoalkaloid content in cooked potatoes (Brown et al. 2008; Bushway et al. 1983; Mattila and Hellström 2007; Mondy and Gosselin 1988; Wu et al. 2004). The impact of cooking on various other fruits and vegetables have also produced mixed results (Bugianesi et al. 2004). Losses in antioxidant activity, carotenoid and phenolic content with cooking have been reported (Craft et al. 1993; Crozier et al. 1997; Ewald et al. 1999; Gil et al. 1999; Ismail et al., 2004; Kuti and Konuru 2004; Roy et al. 2007; Zhang and Hamauzu 2004), while other studies have reported

increases in antioxidant activity, carotenoid and phenolic content (Amakura et al. 2000; Bunea et al. 2008; Choi et al. 2006; Dewanto et al. 2002a; Dewanto et al. 2002b; Dietz et al. 1988; Granado et al. 1992; Huang et al. 2006; Maeda et al. 1992; Miglio et al. 2008; Turkmen et al. 2005; Yamaguchi et al. 2001; Zafrilla et al. 2001). Based on the above listed reports, variability of the impact of cooking on phytochemical content may be related to the type of commodity and genotype, growing location, specific compound assessed, the matrix to which the compound is bound (fats, proteins, carbohydrates, or starches), the amount of physical processing of the fruit or vegetable before cooking, the conditions of the cooking process (including heat-transfer method, time, and amount of water added), and the method of quantification of the compound.

Significant levels of antioxidants, carotenoids and phenolics within cultivars and advanced selections in the Texas Potato Variety Development Program have been identified (Al-Saikhan 1995; Hale 2003; Hale et al. 2008; Nzaramba et al. 2007; Reddivari et al. 2007a; Reddivari et al. 2007b); therefore, the objectives of this study were to determine the effects of storage treatments and cooking methods on total carotenoids (xanthophyll carotenoids, CAR), antioxidant activity (AA), and total phenolic content (TP) in eight cultivars and advanced selections from the Texas Potato Variety Development Program (Atlantic, ATX85404-8W, Innovator, Krantz, NDTX4930-5W, Russet Burbank, Santana, and Shepody) and individual carotenoid and phenolic compounds in three cultivars (Innovator, Russet Burbank, and Santana). These three cultivars were selected because their phenolic content was observed to be consistently high in multiple locations.

Materials and Methods

Plant Materials

Eight named genotypes and advanced selections (Atlantic, ATX85404-8W, Innovator, Krantz, NDTX4930-5W, Russet Burbank, Santana, and Shepody) were grown near Dalhart, in the northwest corner of the Texas Panhandle (planted May, harvested September). The selected clones, with the exception of Santana which has cream flesh, were white-fleshed and were a part of a separate, on-going, multi-year yield, and quality trial. All tubers were harvested in bulk from the field at commercial maturity at a single date and transported to the laboratory for analysis. Tubers were harvested from three separate locations in the field and were analyzed for separate studies on yield and quality parameters, and a random subset of three similar-size tubers from each separate field location was used as replicates for this study. Fresh, non-stored samples were immediately processed, while stored tubers were placed in the various storage conditions.

Processing Methods

Storage treatments Four different storage treatments were conducted on whole tubers, non-stored, 4°C for 110 days, 4°C for 110 days plus 10 days of reconditioning at 20°C, and 20°C for 110 days. The non-stored treatment was an analysis of fresh harvested samples. The other three treatments (4°C, 4°C with reconditioning, and 20°C) involved controlled temperature storage for 110 days in dark, constant-temperature conditions. The reconditioning process involved a secondary treatment after the 110 days of an additional 10 days at 20°C. Potatoes in all storage regimes were stored in single layers in cardboard boxes in dark conditions. Once removed from storage, combined weights of all tubers within a single genotype were compared to their respective weights before storage and a percent weight-loss was calculated. Tubers were visually evaluated for defects and all sprouts were manually removed before further processing.

Cooking Methods Tubers from each field replication were washed, dried and diced into 6.4 mm cubes with a manual vegetable dicer (The Redco Insta Cut 3.5, Lincoln Foodservice, Fort Wayne, IN). Potato peels were not removed prior to dicing. Samples were thoroughly mixed and five (one for each of the four cooking methods and the uncooked control) randomized 5 g samples were taken from each replication. Cubes with skin were homogenized throughout the diced tuber samples. Approximately ten to 15 cubes were utilized for a 5 g sample. Diced tubers were placed in extraction tubes (Falcon, Becton Dickson Labware, Franklin Lakes, NJ) and frozen at -20°C within 15 min of sampling until cooking. Processing times and temperatures for the four cooking times were based on the optimum times and temperatures required to cook an average sample. Preliminary studies determined proper cooking times for each method, which produced a consistent gelatinized starch center determined by hand and mouth-feel, similar to what would be desired by domestic cooking procedures. The uncooked samples remained frozen at -20°C until extraction. After cooking, all cooked samples were frozen at -20°C until extraction.

Baking A gas oven (Montgomery Ward, Cedar Rapids, IA) was brought to 204°C. Samples were cooked for 15 min in glass tubes. After cooking, the samples were removed from the glass tubes and placed back into extraction tubes.

Boiling Water was brought to a boil using a stove range (Montgomery Ward, Cedar Rapids, IA). Ten mL of nanopure, autoclaved water was added to each sample in the extraction tubes. Samples were cooked for 25 min in boiling water in their respective extraction tube. After cooking, the leachate was removed and discarded. The cooked potato samples were patted-dry and placed into extraction tubes.

Frying Canola oil was brought to 191°C in a mini fryer (Rival CF250 Cool Touch Deep Fryer, El Paso, TX). Potato samples were cooked in tea balls for 1 min. After cooking, the samples were removed from the tea balls, patted dry and placed back into extraction tubes.

Microwaving Samples were cooked in their respective extraction tubes for 2.5 min with an 800 W domestic microwave (model MW8985W, Emerson, St. Louis, MO).

Analytical Methods

Extraction of Carotenoids (xanthophyll carotenoids, CAR) The CAR were extracted with methanol with BHT (1 g L⁻¹ for stabilization). Tuber samples (5 g) with extraction solvent (25 mL) were homogenized with an Ultra Turrax Tissumizer T25 (Cincinnati, OH) at 17,500 rpm and centrifuged at 31,000 g for 15 min. The supernatant was collected and analyzed for CAR following the method of Scott (2001). A standard curve for lutein (y=3028.6x+8.1063, R²=0.99) was prepared and used to equate spectrophotometric absorbance readings of the methanol extract at 445 nm to lutein equivalents, where x was the absorbance at 445 nm and y was the µg lutein equivalents per hundred g fresh weight (µg LE 100 g fw⁻¹).

Extraction of Total Phenolics (TP) and Antioxidant activity (AA) For the evaluation of TP and AA, a single extraction was performed. Fifteen mL of methanol was added to a 5 g sample of diced potato. Samples were homogenized with an Ultra Turrax Tissumizer T25 (Cincinnati, OH) at 17,500 rpm and centrifuged at 31,000 g for 20 min. The supernatant was collected for analysis.

DPPH Assay for AA AA was measured using DPPH (2,2 Diphenyl-1-picrylhydrazyl), a colorimetric assay first described by Brand-Williams et al. (1995). The reduction of the DPPH solution is positively correlated with a change in absorbance allowing for a simple assessment of AA. The sample extracts were allowed to react with DPPH until stabilization at 24 h. After this time, the level of reduction was determined by absorbance at 515 nm. A standard curve using a known antioxidant, trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), was prepared, and a regression curve was calculated to convert the change in absorbance into AA. AA was reported as trolox equivalents and determined using the following equation: y=891.69x, where x is the change in absorbance (calculated by subtracting the sample absorbance from the blank of methanol and DPPH at 515 nm), and y was the µg trolox equivalents per g fresh weight (µg TE gfw⁻¹) (R²=0.997).

TP Assay The Folin-Ciocalteu phenol method to determine TP was first described by Swain and Hillis (1959) and modified by Singleton and Rossi (1965). Absorption was determined at 725 nm in plastic UV-spectrophotometeric cuvettes. TP was determined by preparing a regression curve, y=0.5775x-0.0279, where x was the absorbance at 725 nm and y was the µg chlorogenic acid equivalents per g fresh weight (µg CE gfw⁻¹). Chlorogenic acid is one of the most prominent phenolic compounds identified in potato and therefore was chosen as the standard for TP.

HPLC Analysis for Individual Carotenoid and Phenolic Compounds Innovator, Russet Burbank, and Santana were analyzed for individual carotenoid and phenolic compounds; these cultivars were chosen due to previously identified consistently high yield and high CAR, AA, and TP levels in a number of growing locations (data not shown).

The CAR extracted samples (six mL pooled from three replications) were concentrated under nitrogen gas to dryness and the TP extracted samples (six mL pooled from three replications) were dried to completion in a heated speed vac. Samples were resuspended in 0.5 mL of water and 0.5 mL ethanol, filtered separately through a 0.45 µm syringe filter, and injected into a high performance liquid chromatograph (HPLC) for analysis of individual carotenoid and phenolic compounds that had previously been determined as prominent in potato (Hale 2003, Hale et al. 2008; Reddivari et al. 2007a; Lewis et al. 1998). A PC-operated Waters high performance liquid chromatograph (HPLC) system was used to analyze individual carotenoid and phenolic compounds, peaks were identified both by spiking and matching spectra and retention times to those of standards of the carotenoids at 450 nm and the phenolics at 220, 320, and 515 nm. The carotenoids evaluated were antheraxanthin, β -cryptoxanthin, canthaxanthin, lutein, neoxanthin, violaxanthin, and zeaxanthin. All standards were obtained from CaroteNature, Lupsingen, Switzerland and Hoffman La Roche, Bassel, Switzerland. The phenolic compounds evaluated were caffeic acid, chlorogenic acid, (-) epicatechin, ferulic acid, kaempferol, myricetin, p-coumaric acid, quercetin dihydrate, rutin, salicylic acid, syringic acid, t-cinnamic acid, 4,5,7-trihydroxyflavanone, and vanillic acid; all standards were obtained from Agros Organics (Pittsburgh, PA). A nine point standard calibration curve was developed for each compound (Hale 2003). The HPLC system comprised of two binary pumps (Waters 515), an autoinjector (Waters 717 plus), a photodiode detector (Waters 996), and a column heater (SpectraPhysics SP8792) maintained at 35°C for the

carotenoids and 40°C for the phenolics. A 4.6×250 mm. 5 µm, YMC Carotenoid Column (C-30 reverse phase) (Waters, Milford, MA) was used to separate the carotenoids and a 4.6×150 mm, 5 µm, Atlantis C-18 reverse-phase column (Milford, MA) was used to separate the phenolic compounds. The carotenoid detection mobile phases included solvent A: methanol, water, and triethylamine (90:10:0.1), and solvent B: methanol, methyl tert-butyl ether, and triethylamine (6:90:0.1); gradient (min:%A) 0:99, 8:99, 45:0, 50:0, and 53:99 with a flow rate of 1 mL min⁻¹ (Hale 2003; Breithaupt and Bamedi 2002; Reddivari et al., 2007a). The phenolic detection mobile phases included solvent A: 100% acetonitrile and solvent B: nanopure water adjusted to pH 2.3 with HCL; gradient (min:%A) 0:85, 5:85, 30:0, and 35:0 with a flow rate of 1 mL min⁻¹ (Hale 2003; Hale et al. 2008; Reddivari et al. 2007a, Reddivari et al. 2007b).

2) storage treatment, where Russet Burbank uncooked samples were compared; and 3) cooking method, where Russet Burbank, non-stored samples were compared. Analysis of variance (ANOVA) was performed using the general linear model (GLM). CAR, AA, and TP data were analyzed in a full-factorial design and the independent variables included genotype (G), storage treatment (S), cooking method (C), the interactions of G x S, G x C, S x C, and G x S x C. Individual carotenoid and phenolic compounds were only analyzed for the main effects, and the independent variables included genotype (G), storage treatment (S), and cooking method (C). Ranking of the treatment variables was determined using Tukey's HSD Post Hoc analysis. All statistical analyses were performed with SPSS software version 11.5 (SPSS 2002).

Results and Discussion

Identification and Concentration Detected

The phenolic compounds identified and quantified were chlorogenic acid, caffeic acid, rutin, myricetin, vanillic acid, p-coumaric acid, (-) epicatechin, t-cinnamic acid, and quercetin dihydrate, and the only carotenoid detected and quantified was lutein (listed in order of quantity detected) (Table 1). The phenolic compounds—ferulic acid, kaempferol,

Statistical Analysis

A completely randomized block design with three replications was used. Average concentrations of each compound or groups of compounds were determined from an analysis of all samples. Individual parameter ranges were determined for the three main parameters of this study 1) genotype, where non-stored, uncooked samples were compared;

Table 1 Averages, analysis of variance results, and individual factor ranges of antioxidant activity (AA), carotenoid content (CAR), phenolic content (TP), and individual carotenoid and phenolic compounds

Parameter	Overall Average ^a	Effect of genotype		Effect of storage treatment		Effect of cooking method	
		ANOVA Sig. ^b	Range ^c	ANOVA Sig. ^b	Range ^d	ANOVA Sig. ^b	Range ^e
ТР	340±130	**	110–460	**	160-380	**	140–630
AA	310±150	**	88-360	**	190-380	**	210-630
Chlorogenic acid	34±24	*	7.9–13	**	5.7-10	**	8.2-37
Caffeic acid	33±3.2	*	30-30	**	30-32	**	30–39
Rutin	21±8.4	*	5.3-9.8	**	9.8-23.9	*	9.8-32
Myricetin	15±2.2		13-14	*	13-16		14–16
Vanillic acid	$10{\pm}4.2$		0.0-5.9	**	5.9-8.2	**	5.9-15
P-coumaric acid	8.2±1.9		0.0-6.6	**	0.0-7.5	**	0.0-6.5
(-) Epicatechin	$7.0{\pm}3.8$		0.0-6.1		6.1–7.4	*	6.1-7.3
T-cinnamic acid	6.4±1.9	*	6.9–7.0		6.9–6.9		6.9-7.0
Quercetin dihydrate	2.3 ± 2.6	**	0.2-2.0	**	0.0-3.1	**	0.2-1.8
CAR	1.1 ± 0.4	**	0.3-1.6	**	0.9–1.6	**	0.7-1.5
Lutein	$0.1 {\pm} 0.2$		0.0-0.0		0.0-0.3		0.0-0.0

^a CAR is expressed as $\mu g \text{ LE gfw}^{-1}$, AA is expressed as $\mu g \text{ TE gfw}^{-1}$, phenolic content is expressed as $\mu g \text{ CE gfw}^{-1}$ and individual compounds are expressed as $\mu g \text{ gfw}^{-1}$.

^b* Indicates significance at *p*-value<0.05; ** Indicates significance at *p*-value < 0.01

^c Range values are from non-stored, uncooked samples

^dRange values are from Russet Burbank, uncooked samples

^e Range values are from Russet Burbank, non-stored samples

salicylic acid, syringic acid, 4,5,7-trihydroxyflavanone and the carotenoids—antheraxanthin, β-cryptoxanthin, canthaxanthin, neoxanthin, violaxanthin, and zeaxanthin were not detected in any of the three genotypes analyzed. The phenolic acids (chlorogenic acid, caffeic acid, vanillic acid, p-coumaric acid, and t -cinnamic acid) and flavonoids (rutin, myricetin, (-) epicatechin, and guercetin dihydrate) accounted for 67 % and 33 % of the phenolics detected, respectively. Chlorogenic acid was determined to be the most abundant phenolic compound in potato, which has previously been reported (Hale et al., 2008; Hanson and Zucker 1963; Hunter et al. 1957: Reddivari et al. 2007a). The average concentrations of chlorogenic and caffeic acids, 34 and 33 µg/gfw, were within range of previous reports (Hale 2003) and accounted for 37 and 36 % of the phenolic acids, respectively. These percents were similar to those of Lewis et al. (1998) and Mattila and Hellström (2007), but lower than Dao and Friedman (1992). Chlorogenic acid has been reported to be degraded to caffeic acid (Rodriguez de Sotillo et al. 1994), to various unknown compounds (Dao and Friedman 1992), and produce altered HPLC spectra (Mattila and Hellström 2007) in extracted potato samples. Dao and Friedman (1992) presumed that compound may have been an isomer of chlorogenic acid, another phenolic acid associated with the degradation of chlorogenic acid, or a methyl ester formed as a result of esterification of the COOH group on the quinic acid part of chlorogenic acid; however, initial tests could not confirm any of these possibilities. Similar alterations may have occurred in this study, resulting in lower quantified levels than some previous reports, thus under-representing the levels of chlorogenic acid.

Parameter Ranges and Analysis of Variance of Factors

In this study multiple parameters including genotype, storage treatment, and cooking method were analyzed. Ranges of values associated with each factor alone are displayed in Table 1; the range for each parameter was developed by controlling all other factors. The range of values associated with the parameter genotype was developed from non-stored, uncooked samples; the range of values associated with the parameter storage treatment was developed from Russet Burbank, and uncooked samples; and the range of values associated with the parameter cooking method was developed from Russet Burbank, and non-stored samples. Within this analysis, the ranges of TP, AA, chlorogenic acid, caffeic acid, rutin, and vanillic acid were greatest under the column effect of cooking; myricetin, p-coumaric acid, quercetin dihydrate, and lutein were greatest under the column effect of storage; and (-) epicatechin and CAR were highest under the column effect of genotype. The parameter, cooking method produced high variability within a particular genotype; however, the influence of genotype was greater when compared

to cooking method in an analysis of variance proportion. The influence of each parameter, calculated from the proportion of the individual parameter variance to the total of all estimated variance components for CAR were 30 % for genotype, 19 % for storage method, and 3 % for cooking method; for AA were 18 % for genotype, 4 % for storage treatment, and 18 % for cooking method; for TP were 40 % for genotype, 1 % for storage treatment, and 22 % for cooking method.

Analyses of variance of CAR, AA, and TP effects were completed in a full-factorial model of all individual factors and interactions between the individual factors; the individual carotenoid and phenolic compounds were analyzed for only individual factors because replications were pooled for analysis. Each individual factor and the interactions between factors are discussed below in separate sections. Results revealed that all the individual factors: genotype, storage treatments, and cooking methods were significant for CAR, AA, and TP. Chlorogenic acid, caffeic acid, rutin, and quercetin dihydrate exhibited significant main effects for genotype, storage treatment, and cooking method. Vanillic acid and p-coumaric acid also exhibited significant storage treatment and cooking method effects; t-cinnamic acid was the only other individual compound to exhibit significant genotype effects; myricetin was the only other individual compound to exhibit significant storage treatment effects; while (-) epicatechin was the only other individual compound to exhibit significant cooking method effects. Lutein, the only detected individual carotenoid, was not affected by any of the variables analyzed (Table 1). The interaction between genotype and storage treatment (G x S) was significant for CAR, AA, and TP; while the interaction between genotype and cooking method (G x C) and the interaction between storage treatment and cooking method (S x C) was significant for both CAR and TP, as discussed below.

The Effect of Genotype

The effect of the single parameter genotype was determined by comparing between genotypes among all other conditions, including all storage treatments and all cooking treatments and displaying the significant differences among groups of compounds, CAR, AA, TP and individual compounds (Figs. 1 and 2, respectively). From lowest to highest, the range of CAR was 78 (Atlantic) to 140 (Santana) μ g LE 100 gfw⁻¹, while the range for AA was 210 (NDTX4930-5 W) to 420 (Russet Burbank) μ g TE gfw⁻¹ and TP was 210 (NDTX4930-5 W) to 510 (Krantz) μ g CE gfw⁻¹ (Fig. 1). Individual compounds that significantly differed among the three analyzed named genotypes are displayed in Fig. 2; genotype selection influenced 80% of all the quantified phenolic acids (chlorogenic, caffeic, and t-cinnamic acids) and 51% of flavonoids (rutin and quercetin dihydrate).

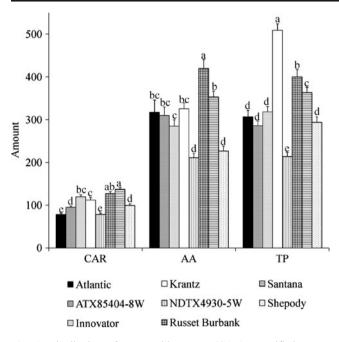


Fig. 1 Distribution of carotenoid content (CAR) quantified as μ g lutein equivalents 100 gfw⁻¹, antioxidant activity (AA) quantified as μ g trolox equivalents gfw⁻¹, and phenolic content (TP) quantified as μ g chlorogenic acid gfw⁻¹ in eight potato genotypes. Each dependent variable was analyzed separately and different lowercase letters indicate significant differences (*p*-value<0.05) between genotypes; while, similar letters indicate no significant differences

The Effect of Storage Treatment

The effect of the single parameter storage treatment was determined by comparing storage treatments among all other conditions, including all genotypes and all cooking

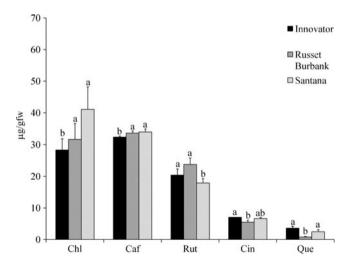


Fig. 2 Distribution of significant individual phenolic compounds: chlorogenic acid (Chl), caffeic acid (Caf), rutin (Rut), T-cinnamic acid (Cin), and quercetin dihydrate (Que) in Innovator, Russet Burbank, and Santana. Each dependent variable was analyzed separately and different lowercase letters indicate significant differences (p-value<0.05) between genotypes; while, similar letters indicate no significant differences

treatments and displaying the significant differences among groups of compounds, CAR, AA, TP and individual compounds (Figs. 3 and 4, respectively). The range for CAR was 79 (non-stored) to 120 (recondition) μ g LE 100 gfw⁻¹, while the range for AA was 280 (20°C) to 360 (4°C) μ g TE gfw⁻¹and TP was 320 (4°C) to 360 (recondition) μ g CE gfw⁻¹(Fig. 3). AA significantly increased with the approximately four-month, 4°C storage treatment when compared to other storage treatments. Similarly, potatoes stored at 5.6°C for 5.5 months also had significantly greater AA when compared to fresh, non-stored samples (Rosenthal and Jansky, 2008).

Individual compounds that significantly differed amongst the storage treatments are displayed in Fig. 4; storage treatments influenced 93 % of all the quantified phenolic acids (chlorogenic acid, caffeic acid, vanillic acid, and p-coumaric acid,) and 84 % of flavonoids (rutin, myricetin, and quercetin dihydrate). The recondition storage treatment produced significantly greater TP, chlorogenic acid, caffeic acid, rutin, vanillic acid, p-coumaric acid, and quercetin dihydrate levels than the non-stored sample; however, no differences in TP, chlorogenic acid, caffeic acid, and vanillic acid were observed for the 4°C storage treatment when compared to the non-stored samples (Figs. 3 and 4). The non-stored samples were lower than all other storage treatments in rutin, p-coumaric acid, and quercetin dihydrate content. In addition, the non-stored samples were lower than the recondition storage treatment samples in chlorogenic acid, caffeic acid, and vanillic acid content, and they were lower than the 20°C and 4°C storage treatments samples in

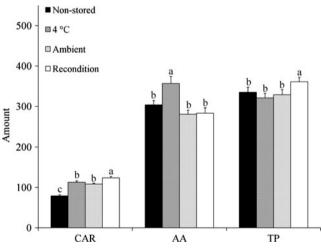


Fig. 3 Distribution of carotenoid content (CAR) quantified as μ g lutein equivalents 100 gfw⁻¹, antioxidant activity (AA) quantified as μ g trolox equivalents gfw⁻¹, and phenolic content (TP) quantified as μ g chlorogenic acid gfw⁻¹ in three storage treatments (time of storage was approximately 4 months) and a non-stored treatment. Each dependent variable was analyzed separately and different lowercase letters indicate significant differences (*p*-value<0.05) between storage treatments; while, similar letters indicate no significant differences

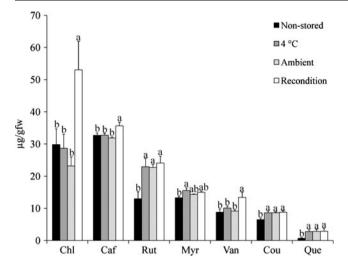


Fig. 4 Distribution of significant individual phenolic compounds: chlorogenic acid (Chl), caffeic acid (Caf), rutin (Rut), myricetin (Myr), vanillic acid (Van), P-coumaric acid (Cou), and quercetin dihydrate (Que) in three storage treatments (time of storage was approximately 4 months) and a non-stored treatment. Each dependent variable was analyzed separately and different lowercase letters indicate significant differences (*p*-value<0.05) between storage treatments; while, similar letters indicate no significant differences

myricetin content. TP, chlorogenic, caffeic, and vanillic acids levels were only positively impacted by the reconditioned storage treatment, while other individual phenolics, rutin, p-coumaric acid, and quercetin dihydrate increased with all storage treatments. Positive correlations have been observed among TP, chlorogenic acid, and caffeic acid (Reddivari et al. 2007a), which may support the similar observed impacts in TP, chlorogenic acid, and caffeic acid under various storage conditions. These results support previous studies that indicate chlorogenic acid is not affected by 20°C temperature storage but may be increased by cold-storage (Hasegawa et al. 1966). In the current study, chlorogenic acid increased during the recondition storage treatment, while 4 or 20°C storage treatments produced no differences. Differences between studies may not be associated with temperature influences but by the injury accumulated during the storage treatment (Johnson and Schaal 1957). Harvest location has also played a critical role in determining the magnitude of phytochemical accumulation during storage (Rosenthal and Jansky 2008). Other confounding variables associated with storage treatment studies include the inherent difference in processing time of the samples.

The level of respiration and the associated loss of carbon, the degree of sprouting, and weight-loss may cause injury during storage (Schippers 1977). Tubers stored at 20°C had sprouted after storage while tubers stored at 4°C or 4°C with reconditioning did not sprout. Combined weights of all tubers within each genotype were compared to their respective weights before storage and a percent weight-loss was calculated for each storage

treatment. Percent weight-loss was determined based on the original fresh weight of the tubers (Table 2). Tubers that were subject to the 20°C storage treatment experienced the greatest weight loss. The genotypes ATX85404-8W, NDTX4930-5W, and Santana lost over 5% weight for all storage treatments. The genotypes with high weight-loss have relatively thin skin, while genotypes with thicker russet skin, Innovator, Krantz, Russet Burbank, lost less weight. Storage treatments with large associated weight-losses were not correlated with increases in phytochemical content. Genotypes that experienced greater weight-loss during storage also did not correlate to any genotype x storage interaction.

The interaction between genotype and storage treatment (Table 3) was significant for CAR, AA, TP, and the influence of this interaction (calculated from the proportion of this interaction variance to the total of all estimated variance components) was 11, 25, and 12%, respectively. In every genotype analyzed, one or more of the storage treatments had higher CAR when compared to non-stored samples. All storage treatments were higher in CAR than non-stored for Atlantic, Krantz, NDTX4930-5W, and Russet Burbank; the recondition and 20°C storage treatments were higher than non-stored for ATX85404-8W and Santana; and the recondition storage treatment was higher than non-stored for Innovator and Shepody. There were no AA differences among storage treatments for five genotypes, Innovator, NDTX4930-5W, Russet Burbank, Santana, and Shepody; and there were no TP differences among storage treatments for four genotypes, ATX85404-8W, Krantz, Santana, and Shepody. In the genotypes where there were differences for AA and TP, one or more storage treatments resulted in greater levels than the non-stored samples. There were two cases where stored samples were not significantly greater than the non-stored: non-stored samples for Krantz were not significantly different in AA levels than any other stored treatment, and non-stored samples for Atlantic were significantly greater in TP than 20°C and recondition storage treatments. Storage treatments largely increased phytochemical content; however, some genotype interactions were observed.

The Effect of Cooking Method

The effect of the single parameter cooking method was determined by comparing the various cooking methods among all other conditions, including all genotypes and all storage methods and displaying the significant differences among groups of compounds, CAR, AA, TP and individual compounds (Figs. 5 and 6, respectively). The variability among cooking methods was much greater for AA (range: 220 μ g TE gfw⁻¹ for uncooked samples to 370 μ g TE gfw⁻¹ for microwaved samples) and TP (range: 260 μ g CE gfw⁻¹ for

Table 2 Distribution of carotenoid content (CAR) quantified as μ g lutein equivalents 100 gfw⁻¹. antioxidant activity (AA) quantified as µg trolox equivalents gfw^{-1} , and phenolic content (TP) quantified as µg chlorogenic acid gfw⁻¹ in the interaction of genotype and storage treatments (time of storage was approximately 4 months). Each dependent variable was analyzed separately and different lowercase letters indicate significant differences (p-value< 0.05) between storage treatments within a single genotype; while, similar letters indicate no significant differences

Genotype	Storage treatment	CAR (μ g lutein equivalents 100 gfw ⁻¹)	AA (μ g trolox equivalents gfw ⁻¹)	TP (μ g chlorogenic acid gfw ⁻¹)
Atlantic	Non-stored	52±13 b	300±93 b	420±96 a
	4°C	86±21 a	650±110 a	360±69 a
	20°C	86±21 a	190±95 c	190±71 b
	Recondition	88±17 a	130±67 c	250±52 b
ATX85404-8 W	Non-stored	80±14 b	270±59 b	290±98 a
	4°C	81±12 b	440±240 a	290±98 a
	20°C	110±19 a	250±60 b	250±75 a
	Recondition	110±21 a	280±110 b	310±48 a
Innovator	Non-stored	99±34 b	310±120 a	240±85 c
	4°C	160±43 a	240±97 a	300±76 bc
	20°C	100±21 b	270±87 a	350±97 ab
	Recondition	120±22 b	320±150 a	380±83 a
Krantz	Non-stored	62±16 b	310±98 ab	490±140 a
	4°C	130±40 a	350±120 ab	540±110 a
	20°C	110±20 a	380±88 a	480±110 a
	Recondition	130±23 a	269±110 b	520±110 a
NDTX4930-5 W	Non-stored	46±14 b	240±86 a	200±64 b
	4°C	91±26 a	200±98 a	180±37 b
	20°C	88±19 a	190±47 a	210±61 b
	Recondition	88±15 a	220±58 a	270±88 a
Russet Burbank	Non-stored	97±22 b	440±130 a	410±130 a
	4°C	140±32 a	380±210 a	290±110 b
	20°C	130±25 a	390±140 a	430±130 a
	Recondition	140±33 a	470±150 a	470±100 a
Santana	Non-stored	120±25 c	360±97 a	350±90 a
	4°C	120±19 c	330±96 a	340±87 a
	20°C	140±19 b	360±86 a	390±120 a
	Recondition	170±22 a	370±140 a	38064 a
Shepody	Non-stored	78±16 b	220±83 a	280±110 a
	4°C	98±36 b	260±120 a	250±53 a
	20°C	100±17 ab	220±40 a	330±120 a
	Recondition	120±25 a	210±99 a	300±110 a

 Table 3 Percent weight-loss for each storage treatment and genotype

	•	-	•	• • •	
Genotype	4°C storage	Recondition storage	20°C storage	Average	
Atlantic	3.4	3.1	8.9	5.1	
ATX85404-8 W	6.5	6.5	11	8.0	
Innovator	2.6	2.5	6.3	3.8	
Krantz	3.8	3.9	6.3	4.7	
NDTX4930-5 W	5.0	5.7	9.4	6.7	
Russet Burbank	3.3	3.3	3.0	3.2	
Santana	6.1	5.8	13	8.3	
Shepody	3.8	4.5	9.8	6.0	
Average	4.3	4.4	8.5	5.7	
-					

boiled samples to 400 μ g TE gfw⁻¹ for microwaved samples) than for CAR (range: 94 µg LE 100 gfw⁻¹ for boiled samples to 110 µg LE 100gfw⁻¹ for fried samples). Boiled samples were lower in CAR than the other samples. Boiled, baked, and uncooked samples were lower in AA and TP than the fried or microwaved samples. Boiling of vegetables causes phenolic constituents to be leached into the cooking water and many phenolics are lost when this water is discarded (Andlauer et al. 2003; Ismail et al. 2004; Zhang and Hamauzu 2004). Greater amounts of phenolics may be extracted out of the potato matrix and into the water used for boiling as compared to the oil used for frying since phenolic compounds are hydrophilic. Individual compounds that significantly differed among cooking methods are displayed in Fig. 6; cooking method influenced 95% of all the quantified phenolic acids (chlorogenic, caffeic, vanillic

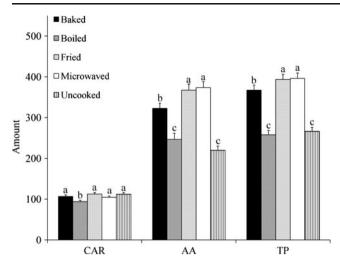


Fig. 5 Distribution of carotenoid content (CAR) quantified as μ g lutein equivalents 100 gfw⁻¹, antioxidant activity (AA) quantified as μ g trolox equivalents gfw⁻¹, and phenolic content (TP) quantified as μ g chlorogenic acid gfw⁻¹ in four cooking methods and an uncooked sample. Each dependent variable was analyzed separately and different lowercase letters indicate significant differences (*p*-value<0.05) between cooking methods; while, similar letters indicate no significant differences

acid, and p-coumaric acid) and 20% of flavonoids ((-) epicatechin and quercetin dihydrate). All cooked samples (baked, boiled, fried, and microwaved) had greater levels of chlorogenic and vanillic acid levels when compared to uncooked samples; in addition, baked, fried, and microwaved samples had greater levels of caffeic acid and p-coumaric acid, and microwaved samples had a higher level of (-) epicatechin when compared to the uncooked samples. This study supports numerous other reports (Bunea et al. 2008; Choi et al. 2006; Dewanto et al. 2002a; Dewanto et al. 2002b; Huang et al. 2006; Klein and Kurilich 2000; Maeda et al. 1992; Mattila and Hellström 2007, Miglio et al. 2008; Ranilla et al. 2009; Sultana et al. 2008; Turkmen et al. 2005; Wu et al. 2004; Yamaguchi et al. 2001) where AA and TP increased in starchy, cooked fruits and vegetables when compared to uncooked samples. It is assumed, that improved extractability of the AA and phenolics compounds from the cellular matrix of cooked samples is responsible for the increase. Improvements in the ability to extract chlorogenic acid in potato under heat processes were also observed by Reeve et al. (1969); yields of chlorogenic acid levels nearly quadrupled in an ethanol extraction that was boiled as compared to ethanol Soxhlet extraction of freeze-dried potato. Miglio et al. (2008) strengthened this assumption by associating shear force and percent softening to increases in glucosinolates, carotenoids, and AA in cooked samples of broccoli, carrots, and courgettes.

Potato peels have previously been shown to contain a high quantity of phenolics (Dao and Friedman 1992; Mondy and Gosselin 1988; Reeve et al. 1969) and the removal of the peel before or after cooking appears to be influential in determining phytochemical levels. Mattila and Hellström (2007) observed a decrease in levels of phenolics in peeled and cooked potato when compared to uncooked samples; conversely, cooked potato peels of similar genotypes had enhanced phenolic levels when compared to uncooked peels. Mondy and Gosselin (1988) suspected that during the cooking process phenolics migrated from the peel into both the cortex and internal tissues of the potato. Potatoes were cooked with or without a peel and tubers that were cooked with the presence of an intact peel had a greater amount of total phenols in the cortex and internal tissues when compared to uncooked samples. In a similar study involving sweet potato flours, Huang et al. (2006) also suggested that there was an improved extractability of phenolics from cooked samples. In this 2006 study, both cooked and uncooked samples were analyzed without peels; however, the cooked samples were cooked with intact peels and then peeled.

Not all the phenolic compounds behaved similarly in the present study, quercetin dihydrate was the only compound that was negatively impacted by all cooking methods when compared to the uncooked sample. Similar decreases in levels of quercetin derivatives have previously been noted during thermal treatments in both fruits and vegetables, including potato (Crozier et al. 1997; Ewald et al. 1999; Häkkinen et al. 2000; Tudela et al. 2002).

The interaction between genotype and cooking method and the interaction between storage treatment and cooking method were significant for CAR and TP; however, all their

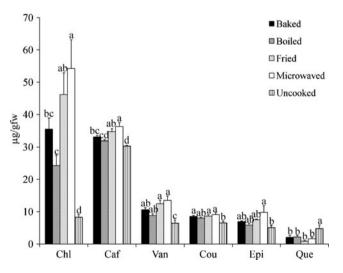


Fig. 6 Distribution of significant individual phenolic compounds: Chlorogenic acid (Chl), caffeic acid (Caf), vanillic acid (Van), P-coumaric acid (Cou), (-) epicatechin (Epi), and quercetin dihydrate (Que) in four cooking methods and an uncooked samples. Each dependent variable was analyzed separately and different lowercase letters indicate significant differences (*p*-value<0.05) between cooking methods; while, similar letters indicate no significant differences

respective influences were 5% or below. The interaction between all three parameters, genotype, storage treatment, and cooking method, was not significant for any dependent variable. There were only two genotypes, Innovator and Russet Burbank, where CAR significantly differed among cooking methods. In both genotypes, boiled samples were lower than other cooking methods, fried (Innovator) and baked (Russet Burbank). In the genotype, Atlantic, cooking methods did not differ in TP. For the other analyzed genotypes, fried samples resulted in greater TP than uncooked samples (ATX85404-8W); fried and microwaved samples resulted in greater TP than uncooked and boiled (Shepody) and for the remaining genotypes baked, fried and microwaved samples resulted in greater TP than both uncooked and boiled samples.

There was one storage treatment, 4°C, where CAR differed among cooking methods: for tubers that were stored at 4°C, fried samples were greater in CAR than boiled samples. Among all storage treatments, one or more cooking methods resulted in greater levels of TP than both the boiled and uncooked samples. In the recondition treatment, microwaved samples were greater in TP levels than the boiled and uncooked samples; and in the remaining storage treatments, the baked, fried, and microwaved samples resulted in greater TP levels than boiled and uncooked samples. The interaction between genotype and cooking method and the interaction of storage treatment and cooking method were significant for the determination of CAR and TP; however, CAR and TP within these two interactions were affected similarly to the observed effects of the single parameter, cooking method.

Potato's Contribution to a Healthy Diet

High concentrations of phenolics and antioxidants have been associated with fruit species especially berries; however, significant levels of phytochemicals and antioxidants are also found in potatoes (Wu et al. 2004). The specific compounds analyzed in this study in potato were compared to those found in blueberry (data not shown). Blueberry contains higher levels of some phenolic compounds (chlorogenic acid: 110 µg gfw^{-1} in blueberry and 34 µg gfw^{-1} in potato); while potato contains higher levels of some carotenoids (lutein: <1 µg 100 gfw⁻¹ in blueberry and 11 μ g 100 gfw⁻¹ in potato). The 2008 US average per capita availability (adjusted for loss), an estimate for average per capita consumption, of potatoes and blueberries is 54,000 g and 540 g, respectively (USDA/ERS, 2010). Thus, Americans consume over 32 and 1,100 times greater chlorogenic acid and lutein from potato than blueberry. Potato is a major contributor to consumed phytochemical levels in the American diet.

Genotype selection has previously been identified as a significant factor in controlling antioxidant and phytochemical

levels in potato. This study has identified postharvest processing techniques, including storage and cooking, as influential factors in describing these levels. The majority of potato consumption involves these two postharvest processing techniques. Storage of potatoes increased CAR, AA, TP and individual phenolic levels; similar trends were observed when storage treatments were analyzed per genotype (the interaction of genotype by storage); however only about half of the analyzed genotypes were affected by storage treatments for AA and TP.

The cooking methods, baking, frying, and microwaving increased AA, TP, and most individual phenolic compounds when compared to uncooked samples. This phenomenon is believed to be associated with an increase in extractability of these compounds from the potato's cellular matrix due to starch textural changes during the cooking processes. Future studies should address the bioavailability of potato phenolics after various cooking methods.

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