Food Chemistry 127 (2011) 34–41

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

HPLC profiling of phenolics in diverse potato genotypes

Duroy A. Navarre [a,b,*], Syamkumar S. Pillai [b], Roshani Shakya [b], Margaret J. Holden [a]

[a] USDA-Agricultural Research Service, Washington State University, 24106 N. Bunn Rd., Prosser, WA 99350, United States
[b] Irrigated Agriculture and Extension Research Center, Washington State University, United States

ARTICLE INFO

Article history:
Received 24 July 2010
Received in revised form 19 November 2010
Accepted 16 December 2010
Available online 23 December 2010

Keywords:
Phytonutrients
Phenolics
Antioxidants
Flavonols
Chlorogenic acid
Potato
Solanum tuberosum L.
Wild-species

ABSTRACT

Potatoes from over fifty genotypes representing cultivars, breeding lines, primitive germplasm and wild species were analysed for phenolic content and hydrophilic antioxidant capacity. Genotypes with markedly higher amounts than the most commonly consumed potatoes were identified. Chlorogenic acid was the most abundant phenolic and ranged from 22 to 473 mg/100 g dry weight. Rutin and kaempferol-3-rutinose were the most abundant flavonols. Total phenolics ranged from 1.8 to 11 mg/g DW and antioxidant capacity from 27 to 219 μmol TE/g DW. Total phenolics and antioxidants in these high-phytonutrient potatoes compared favourably to 15 other analysed vegetables. With the high per capita consumption of potatoes, widespread adoption of high-phytonutrient cultivars could significantly increase dietary intake of phytonutrients.

Published by Elsevier Ltd.

1. Introduction

Staple crops are primary targets for biofortification because even modestly increasing their phytonutrients would result in a significant increase in dietary intake given their high consumption. Potatoes are the most consumed vegetable in the United States and much of the developed world, and consumption is increasing in the developing world (FAO). Potatoes have significant genetic diversity that can be utilised for biofortification, can be cultivated in a wide geographical range, and are an energy-efficient crop that produces a high amount of calories per acre, more than rice or wheat (Knorr, 1977). The latter trait may increase in importance given the competition for arable land and challenge of meeting the caloric and nutritional needs of a growing global population projected by the United Nations to reach seven billion in 2012.

Another rationale for developing high-phytonutrient staple crops is that the nutritional merits of some, such as rice and potatoes, have been criticised with recommendations that these foods be eaten sparingly (Willett & Stampfer, 2003). One study reported a modest association between potato consumption and risk of type 2 diabetes in women (Halton et al., 2006).

Over the last decade, various groups have worked on enhancing the phytonutrient content of potatoes and demonstrated the nutritional potential of the crop. Several groups (Bub, Möseneder, Wenzel, Rechkemmer, & Briviba, 2008; Ducreux et al., 2005) have used transgenic approaches to increase potato carotenoids, including one strategy that increased β-carotene 3600-fold to 47 μg/g DW (Diretto et al., 2007), whereas a survey of Andean germplasm identified genotypes with carotenoid concentrations up to 36 μg/g DW and some with 2 μg/g DW of β-carotene, a carotenoid not typically found in significant amounts in potatoes (Andre et al., 2007a, 2007b). Screening Andean germplasm identified several high-phenolic lines (Andre et al., 2007b), whereas overexpressing a MYB transcription factor upregulated tuber phenylpropanoid metabolism resulting in significant increases in chlorogenic acid, flavonols and anthocyanins (Rommens et al., 2008). An anthocyanin-rich fraction from potatoes showed anti-cancer properties in a cell culture assay (Reddivari, Vanamala, Chintharlapalli, Safe, & Miller, 2007) and extracts from red-pigmented potatoes inhibited carcinogenesis in rats (Thompson et al., 2009). Additional opportunities to enhance tuber anthocyanin content are suggested by a transcriptome analysis that identified 27 genes differentially regulated between white and purple tissue (Stushnoff et al., 2010). Metabolomics have been used to analyse GM potatoes and characterise diversity in native germplasm (Catchpole et al., 2005; Dobson et al., 2010).

The USDA databases (USDA National Nutrient Database SR 22) provide data on only a few potatoes; so it is informative to compare the phytonutrient content of less widely planted cultivars that have never been characterised to the mainstream varieties. Such

* Corresponding author. Tel.: +1 509 786 9261; fax: +1 509 786 9370.
E-mail address: roy.navarre@ars.usda.gov (D.A. Navarre).

0308-8146/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2010.12.080

information can clarify what reasonable targets are for increasing potato phytonutrients by breeding. In this study we characterised phenolics and antioxidant capacity among potato genotypes and examined how the high-phytonutrient genotypes identified compared to some other vegetables.

2. Materials and methods

2.1. Chemicals

2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma Aldrich and fluorescein diacetate was obtained from Fluka Chemie. Stock solutions of fluorescein (5 mM in 75 mM phosphate buffer, pH 7.0) and 10 mM 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) in 75 mM phosphate buffer were divided into smaller aliquots and stored at −80 °C. A 75 mM 2,2′-Azobis-2-methyl-propanimidamide, dihydrochloride (AAPH) solution in 75 mM phosphate buffer, pH 7.0 was prepared fresh. EDTA and HPLC grade methanol were purchased from Fisher (NJ). Acetonitrile, acetic acid, formic acid, and trifluoroacetic acid (TFA) were purchased from J.T. Baker (NJ). Ammonium formate was from Fluka. Chlorogenic acid, caffeic acid, dl-tyrosine, dl-tryptophan, chaconine and solanine were purchased from Sigma (St. Louis, MO). Kaempferol was purchased from MP Biomedicals (Solon, OH) and rutin trihydrate from Alfa Aesar (Ward Hill, MA). All standards were prepared as stock solutions at 10 mg/ml in methanol except tyrosine and tryptophan, which were prepared in 0.1 N HCl, and solanine, which was prepared in 2.5% metaphosphoric acid.

2.2. Plant material and sample preparation

Tubers from cultivars and advanced breeding lines (*Solanum tuberosum* L.) were obtained from fields at Hermiston, OR; Patterson, WA; Pullman, WA; Othello, WA, and Wasilla, AK, during the years 2003–2007. Wild species (*S. bulbocastanum*, PI243510; *S. chacoense*, PI275139; *S. commersonii*, PI472837; *S. jamesii*, PI275262; *S. kurtzianum*, PI472923; Huagalina, PI 642180; *S. pinnatisectum*, PI184774; *S. spegazzini*, PI205407; *S. stenotomum*, PI195204; *S. tarijense,* PI195206; Paciencia, PI 642187) were obtained from NSRP-6 (www.ars-grin.gov/) in Sturgeon Bay, WI. For each tuber, three slices (one upper third, one middle, one lower third, obtained longitudinally and containing flesh and skin) were ground in liquid nitrogen immediately after slicing, freeze-dried, and stored at −80 °C until analysis. Because of the small tuber size of wild species and immature potatoes, whole tubers were used. Immature potatoes weighing about 28 g and mature tubers were used from the genotype CO79226-2 (immature tubers are differentiated by an asterisk.) Fresh vegetables (green bell peppers, broccoli, green cabbage, yellow corn, green lettuce, peas, scallions, Roma tomatoes, zucchini, green beans, white cauliflower, orange carrots, spinach, eggplant and Brussels sprouts) were purchased from a local supermarket and freeze-dried. The green leaves of the scallions were used, florets for broccoli and cauliflower, whole pods for peas, and the seeds removed from green bell peppers. For the leafy vegetables, the outer 2–3 layers were removed prior to processing. Three independent biological replicates were used for each potato genotype or vegetable in both the HPLC and ORAC analyses.

Freeze-dried powder (typically 200 mg for white potatoes and 100 mg for coloured potatoes and other vegetables) was placed into a 2 ml screwcap tube along with 0.9 ml of extraction buffer (50% MeOH, 2.5% metaphosphoric acid, 1 mM EDTA) and 500 mg of 1.0 mm glass beads and shaken in a BeadBeater (Biospec, Bartelsville, OK) for 15 min at maximum speed, then centrifuged for 5 min at 4 °C, and the supernatant transferred to a clean tube. The remaining pellet was reextracted with 0.6 ml of extraction buffer and centrifuged. The supernatants were combined, centrifuged, and concentrated in a Speed Vac (Thermo Savant, Waltham, MA) prior to HPLC analysis. Samples and solutions were kept chilled at all times and not exposed to bright light.

2.3. Phenolic and antioxidant assays

Total phenolics of three biological replicates per genotype were measured using Folin–Ciocalteu (FC) reagent in a modified protocol of (Skerget et al., 2005). Hundred microlitres of the extract was pipetted into a 4.5 ml cuvette and 1.5 ml water added with 100 μl FC reagent, mixed well and incubated for 3 min at room temperature. After adding 300 μl of 20% Na_2CO_3 the sample was incubated 30 min at 40 °C and absorbance was measured at 755 nm. ORAC analyses were carried out in a Gemini XPS spectrofluorometer (Spectromax Gemini XPS, Molecular Devices Corporation, California, USA) with a dual scanning microplate reader with an excitation wavelength of 485 nm and an emission wavelength of 530 nm. The plate reader was controlled by Softmax Pro software. The oxygen radical absorbance capacity was determined as described by (Gillespie, Chae, & Ainsworth, 2007) with slight modifications. The reaction was carried out in 75 mM sodium phosphate buffer (pH 7.0), and the final reaction mixture was 200 μl. The mixture was pre-incubated for 10 min at 37 °C, before adding the AAPH solution (25 μl) and fluorescence recorded every 2 min for 120 min. A single dilution of each sample was used, with the dilution based on the total phenolic content and preliminary experiments to identify dilutions that gave readings within the standard curve range. Three biological replicates were analysed with three technical replicates each. Standard deviation was calculated from the means of pooled technical replicates.

2.4. LCMS parameters

Analysis was conducted using an Agilent 1100 HPLC system with a quaternary pump, refrigerated autosampler, column heater and DAD and MS detectors. Flow rate was 1 ml/min and injection volume was 5 μl. Column temperature was 35 °C. Two different columns were used, one a 100 × 4.6 mm, Onyx monolithic C-18 (Phenomenex) column at a flow rate of 1 ml/min with a gradient elution of 0–1 min 100% A, 1–9 min 0–30% B, 9–10.5 min 30% B, 10.5–14 min 35–65% B, 14–16 min at 65–100% B, 16–16.5 min 100% B (Buffer A: 10 mM formic acid pH 3.5 with NH_4OH; Buffer B: 100% methanol with 5 mM ammonium formate). A 3 × 150 mm, 3.5 μm Zorbax Eclipse XDB C-18 (Agilent) was used at a flow rate of 1 ml/min with a gradient elution of 0–1.5 min, 0–4% B; 1.5–5 min, 10–25% B; 5–10 min, 35–65% B; 10–15 min, 70–90% B; 15–16 min, 100% B and same mobile phases as above. MS analysis was with an Agilent 1100 LC/MSD VL or SL ion trap using an ESI source in both positive and negative ion mode. The source was operated using 350 °C drying gas (N2) at 12 l/min, 55 psi nebulizer gas (N2), and the source voltage with a scan range of *m/z* 100–1300. Automated MS^2 fragmentation was conducted using SmartFrag software (Agilent) and ramped CID voltage of 1500–4500 and data analysed using Agilent ChemStation software. The external standard method of calibration was used. Neochlorogenic and cryptochlorogenic acids were quantitated as chlorogenic acid equivalents and kaempferol-3-O-rutinoside as rutin equivalents. Kaempferol–rutinose standards were not available, so tentative identification was based on the UV spectrum and a pseudomolecular ion of *m/z* 593 $(M-H)^-$ and a MS^2 product ion of *m/z* 285 $(M-H-\text{rutinose})^-$.