JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Developmental Effects on Phenolic, Flavonol, Anthocyanin, and Carotenoid Metabolites and Gene Expression in Potatoes

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Supporting Information

ABSTRACT: Potato phytonutrients include phenolic acids, flavonols, anthocyanins, and carotenoids. Developmental effects on phytonutrient concentrations and gene expression were studied in white, yellow, and purple potatoes. Purple potatoes contained the most total phenolics, which decreased during development (from 14 to 10 mg g^{-1}), as did the activity of phenylalanine ammonia-lyase. The major phenolic, 5-chlorogenic acid (5CGA), decreased during development in all cultivars. Products of later branches of the phenylpropanoid pathway also decreased, including quercetin 3-*O* rutinoside, kaempferol 3-*O*-rutinoside, and petunidin 3-*O*-(*p*-coumaroyl)rutinoside-3-glucoside (from 6.4 to 4.0 mg g^{-1}). Violaxanthin and lutein were the two most abundant carotenoids and decreased 30−70% in the yellow and white potatoes. Sucrose, which can regulate phenylpropanoid metabolism, decreased with development in all cultivars and was highest in purple potatoes. Total protein decreased by 15−30% in two cultivars. Expression of most phenylpropanoid and carotenoid structural genes decreased during development. Immature potatoes like those used in this study are marketed as "baby potatoes", and the greater amounts of these dietarily desirable compounds may appeal to health-conscious consumers.

KEYWORDS: *potato, chlorogenic acid, flavonols, anthocyanins, carotenoids, sucrose, tuber development, gene expression, health*

INTRODUCTION

The biosynthesis of plant secondary metabolites, many of which are phytonutrients, is under genetic control and also influenced by environmental factors.[1,2] Potato is an important tuber crop and the most consumed vegetable in the developed world. Potatoes can contain significant amounts of phenylpropanoids, the type and abundance of which vary depending on the genotype.[3,4] 5-Chlorogenic acid (5CGA) is the major phenolic compound in most cultivars,[5] and anthocyanins are present in red and purple cultivars.[6] Carotenoids are a different class of phytonutrients that accumulate to higher levels in yellow genotypes.[7] Sucrose and hormones such as jasmonic acid, abscisic acid, and ethylene can induce phenylpropanoid expression,[8,9] as can longer days and cooler temperatures.[10] Phenolics, anthocyanins, and carotenoids can have antioxidant activity and benefit human health.[11,12] Some of these metabolites have anti-inflammatory properties and may have the potential to reduce cardiovascular diseases, macular degeneration, and severity of cataracts.[13,14]

The tuber life cycle includes induction, initiation, and enlargement.[15] Tuber development is regulated by endogenous factors such as sucrose, hormones, and polyamines[16,17] and environmental factors including day length and temperature.[17] The developmental stage can influence tuber phytonutrient content. Higher concentrations of carotenoids were found in swelling stolons and developing tubers than in mature tubers.[18] A decrease was observed in folate levels in tuber flesh and anthocyanins in tuber periderm during tuber development.[19,20] Developmental effects on potato phytonutrients have not been well studied in the latter stages of development in field-grown tubers.

A previous study of white potatoes found significant changes in hydroxycinnamic acids during development that correlated with changes in phenylpropanoid gene expression and sucrose concentrations.[32] Immature potatoes had higher amounts, a finding that has nutritional significance because such potatoes are marketed as "new" or "baby" potatoes. Not determined in the earlier study was whether later branches of the phenylpropanoid pathway leading to flavonoids and anthocyanins were also modulated by development, or if there were developmental effects on other nutritionally important compounds such as carotenoids and protein. If immature tubers have higher amounts of other phytonutrients in addition to hydroxycinnamic acids, then this would further increase the nutritional significance of developmental effects and the merits of "baby potatoes". To address such questions, a comprehensive analysis of developmental effects on numerous phytonutrients was

Received: April 5, 2013
Revised: June 20, 2013
Accepted: June 21, 2013
Published: June 21, 2013

conducted that measured metabolite and transcript abundance in field-grown potatoes with white, yellow, or purple flesh.

MATERIALS AND METHODS

Plant Materials. Three potato cultivars, Green Mountain (GM), Keuka Gold (KG), and Magic Molly (MM), were grown in Fairbanks, AK (latitude, 64° 51′ 33″ N; longitude, 147° 49′ 29″ W), in 2007 and 2008 in 91 cm rows, with 28 cm spacing, 8−10 cm deep. Certified seed of each cultivar was obtained from Cornell University. No chemical destruction of the haulms was used, and potatoes were harvested in 10 day intervals once tubers reached the bulking stage. Tubers from different plants were pooled to give independent biological triplicates. A single plant can contain a range of tuber size distributions that reflect different levels of maturity. The mean weight of tubers analyzed at each developmental time point is shown (Supporting Information, Supplemental Figure 1). Multiple 2 mm slices were collected from the top, middle, and bottom (longitudinally) of the tubers to ensure even representation and avoid tuber metabolic gradients. Slices included the periderm around the periphery only, but not the top or bottom of the slices. Tuber slices were immediately frozen in liquid nitrogen, freeze-dried, and stored at −80 °C until used.

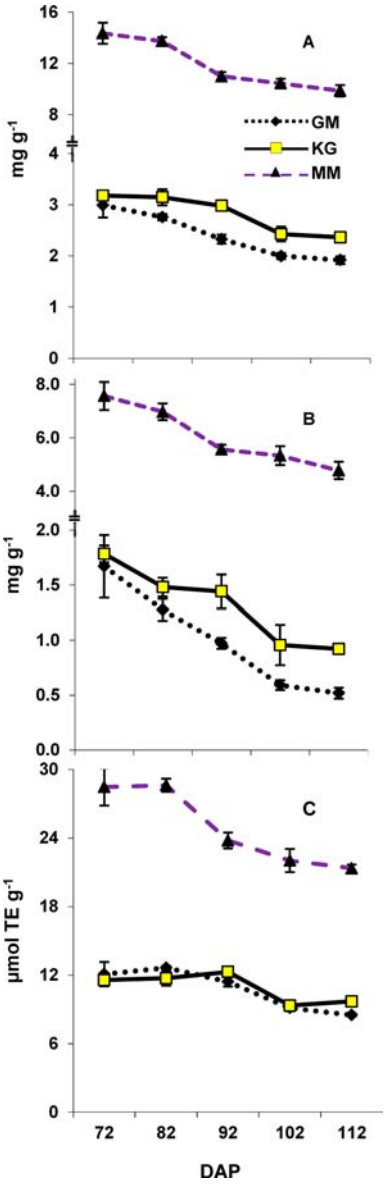

Figure 1. Amounts of total phenolics (A), chlorogenic acid (B), and antioxidant capacity (C) during tuber development in white, yellow, and purple potatoes. DAP, days after planting; GM, Green Mountain; KG, Keuka Gold; MM, Magic Molly. The data represent the mean ± SE (n = 3). A break in the Y-axis indicates discontinuous scale.

Phenylpropanoid Analysis. Phenolics were extracted with 50% MeOH containing 2.5% metaphosphoric acid and 1 mM EDTA.[21] To either 50 mg (MM) or 100 mg (GM and KG) of dried sample were added 900 μL of buffer and 500 mg of 1 mm glass beads, and the mixture was agitated for 5 min in a BeadBeater (Biospec, Bartelsville, OK, USA). After a 5 min centrifugation, the pellet was reextracted with 600 μL of buffer, and the supernatants were combined. Total phenolics were estimated by Folin−Ciocalteu (FC) reagent as gallic acid equivalents.[22] Individual phenolics were determined by LC-MS.[21]

Anthocyanins were extracted from 50 mg of freeze-dried powder with 1.5 mL of 50% methanol and 2.5% formic acid and identified and quantitated using LC-MS.[2] LC-MS data were used for compound identification (Supporting Information, Supplemental Table 1).

For phenylalanine ammonia-lyase (PAL) enzyme assays, soluble proteins from 50 mg of tuber sample were extracted with 50 mM Tris-Cl (pH 8.8) containing 1 mM EDTA, 1 mM PMSF, 5.7 mM 2-sulfhydrylethanol, 1% insoluble PVPP, and 0.2% Triton X-100. PAL activity was determined by measuring the cinnamic acid formed and reported as nanomoles of cinnamic acid per milligram of protein per minute.[23] Antioxidants were determined in an aliquot of 30 μL (for GM and KG) or 15 μL (for MM) phenolic extract with 1 mL of FRAP reagent.[24] Solutions were incubated for 5 min at 37 °C and centrifuged for 1 min, and the absorbance was read at 593 nm; antioxidant values were calculated as trolox equivalents. For lignin analysis, the pellet remaining after sugar extraction was dissolved in 10% thioglycolic acid (1.8 mL, Sigma) in 2 M HCl and heated at 95 °C for 4 h. After centrifuging, the pellet was dissolved in 0.5 M NaOH (1.5 mL) and incubated with shaking for 18 h at room temperature. Lignin from the supernatant was pelleted by adding concentrated HCl (0.5 mL). The pellet was dissolved in 0.5 M NaOH. Lignin was quantified by substituting A_{280} values in the equation derived from dried bamboo milled wood lignin.[25]

Carotenoids. Carotenoids were extracted from 100 mg of tuber powder with 300 μL of methanol (containing 200 ng of β-apo-caroten-8-al as internal standard) and 300 μL of Tris-HCl (50 mM, pH 7.5, 1 M NaCl), after which 800 μL of chloroform was added, vortexed, and centrifuged for 5 min. The aqueous phase was reextracted with chloroform, and the two chloroform extracts were combined and dried in a SpeedVac. The pellet was redissolved in 70 μL of methanol/ethyl acetate (4:1) containing 0.1% butylated hydroxytoluene, and 30 μL was injected onto an Agilent 1100 HPLC.[2] All carotenoids were quantified at 450 nm as lutein equivalents and identified by their retention time and spectral characters.[26,27]

Analysis of Gene Expression. RNA was extracted from a 50 mg sample with 1 mL of hot CTAB buffer (65 °C, 2% w/v cetyltrimethylammonium bromide, 1.4 M NaCl, 20 mM EDTA, 0.1 M Tris-HCl, pH 8.0, 2% w/v PVP (K-30), 0.2% v/v β-mercaptoethanol). Extracts were treated with acidified phenol/chloroform/isoamyl alcohol (125:24:1), followed by chloroform/isoamyl alcohol extraction. RNA was collected after LiCl precipitation. The pellet was dissolved in 300 μL of water, then precipitated with 30 μL of sodium acetate (3 M, pH 5.2) and 750 μL of 95% EtOH, and washed with 70% EtOH. The quality and quantity were assayed in a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and on an agarose gel. cDNA was synthesized from 2 μg of total RNA, by M-MuLV reverse transcriptase (New England BioLabs) after incubation at 42 °C for 2 h. qRT-PCR was performed in 384-well plates from 4 ng of RNA equivalent cDNA, 1× SYBR Green Mix (Roche, Mannheim, Germany), and 400 nM primers in a LightCycler 480 (Roche). Relative gene expression was calculated by the deltaCT method[28] using elongation factor 1-α (EF1- α) and actin for template normalization and primers previously described.[2,4,29]

Sugars and Protein Analysis. Soluble sugars were extracted from 25 mg of dry sample with 2 mL of 80% ethanol by heating at 80 °C for 15 min. After reextraction with 50 mg of activated charcoal, an aliquot of the extract was dried completely, and the pellet was dissolved in

water and used for sucrose and glucose estimation using Sigma kits (SCA20, GAHK20). Total nitrogen content was estimated in 10 mg of powder by dry combustion (CNS analyzer, Vario EL Elemental, Elementar Americas, Mt. Laurel, NJ, USA). The total protein was then estimated by multiplying the percent of nitrogen content by 6.25.[30]

RESULTS AND DISCUSSION

Changes in phytonutrients during tuber development were measured in potatoes from white- (GM), yellow- (KG), and purple-flesh (MM) potatoes. Relative to white potatoes, yellow potatoes are expected to have higher amounts of carotenoids and purple potatoes more anthocyanins; MM mature tubers were previously found to have high amounts of antioxidants.[21] Tubers were collected starting from early bulking through the end of the growing season, spanning a range of 72−112 days after planting (DAP). Field-grown potatoes were used to ensure results would be representative of the actual crop, because data obtained using greenhouse potatoes can be markedly different from those obtained with cropped potatoes.[31,32] From a nutritional perspective, it should be noted that all of the developmental stages used in this study represent sizes of potatoes that are commonly consumed.

General and Early Phenylpropanoid Metabolism. The purple potatoes had the highest amounts of phenolics (14 mg g^{-1} DW), whereas yellow and white had lower amounts (3 mg g^{-1} DW, Figure 1A). Depending on the cultivar, total phenolics decreased 26−36% during tuber development. Chlorogenic acid (5CGA) and four isomers were identified by LC-MS. 5CGA was the most abundant form, followed by 4-chlorogenic acid (4CGA) and *cis*-5CGA (Table 1). Total CGAs in immature potatoes were 7.5 mg g^{-1} in MM and ~1.7 mg g^{-1} in KG and GM. 5CGA comprised >95% of the total CGAs in MM and between 80 and 90% in KG and GM. Total CGAs and 5CGA concentrations decreased during development in all cultivars (Figure 1B and Table 1). The developmental decrease in 5CGA ranged from 72% in GM to 39% in MM. Phenylpropanoids, especially CGAs, are important antioxidants in potatoes. The antioxidant capacity of 28 μmol TE g^{-1} in the high-phenylpropanoid cultivar MM was 2.5-fold higher than in KG and GM (Figure 1C) and decreased during tuber development. Similar trends were observed for seven other hydroxycinnamic acid derivatives, including hydroxycinnamic acid amides (Supporting Information, Supplemental Table 2).

Concentrations of the three shikimic acid derived aromatic amino acids, tyrosine, phenylalanine, and tryptophan, varied among cultivars and did not consistently show tendencies to decrease during development (Supporting Information, Supplemental Table 3). Phenylalanine is the substrate for PAL, which catalyzes the first committed step in phenylpropanoid biosynthesis. Interestingly, phenylalanine concentrations were 2−3-fold lower in the high-phenylpropanoid purple potatoes compared to white or yellow. PAL activity was >10-fold higher in MM than in the other two genotypes and reached 1.6 nmol cinnamic acid mg^{-1} protein min^{-1}. During tuber development, PAL enzyme activity decreased markedly in all cultivars (Figure 2).

Late Phenylpropanoid Metabolism: Lignin, Flavonols, and Anthocyanins. The above data showed developmental effects on tuber hydroxycinnamic acid metabolism, but do not reveal whether biosynthetic branches later in the phenylpropanoid pathway are developmentally regulated. Two previous studies reported a decrease in total anthocyanins during tuber development,[10,20] but the present study differs by providing more detail about overall phenylpropanoid metabolism, individual compounds and phenylpropanoid gene expression. In addition to the soluble phenolics described above, insoluble lignin is a major product of phenylpropanoid biosynthesis. However, unlike the soluble phenylpropanoids, no obvious trend in lignin concentrations was seen during development (Figure 3).

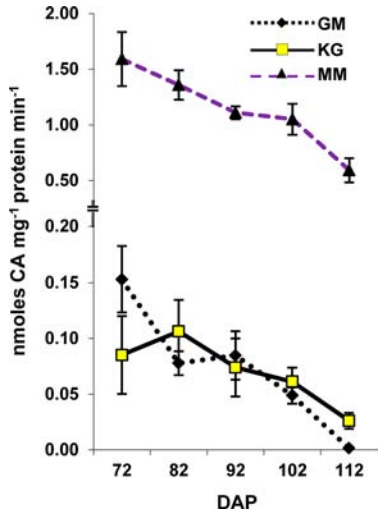

Figure 2. Changes in PAL enzyme activity during tuber development in white, yellow, and purple potatoes. GM, Green Mountain; KG, Keuka Gold; MM, Magic Molly. The data represent the mean ± SE (n = 3). A break in the Y-axis indicates discontinuous scale.

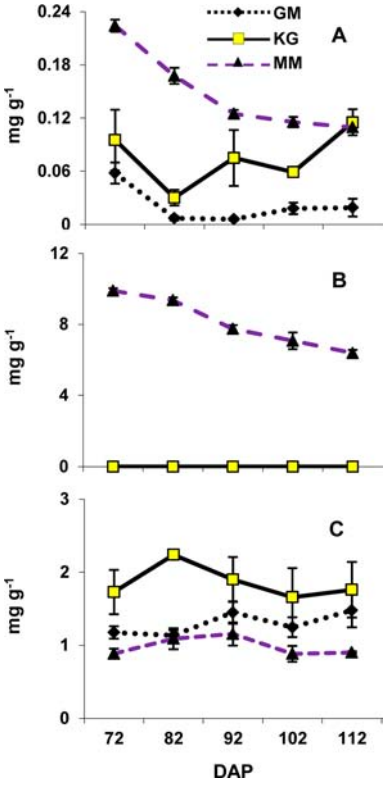

Figure 3. Amounts of total flavonols (A), anthocyanins (B), and lignin (C) during tuber development in three genotypes. GM, Green Mountain; KG, Keuka Gold; MM, Magic Molly. The data represent the mean ± SE (n = 3).

dx.doi.org/10.1021/jf401522k | J. Agric. Food Chem. 2013, 61, 7357−7365

Table 1. Concentrations of Chlorogenic Acids[a] during Five Stages of Tuber Development in White, Yellow, and Purple Potatoes[b]

genotype[c]	DAP[d]	5CGA (mg g^{-1})	4CGA (mg g^{-1})	*cis*-5CGA (mg g^{-1})	3CGA (mg g^{-1})	*cis*-4CGA (μg g^{-1})
GM	72	1.548 ± 0.268	0.099 ± 0.015	0.019 ± 0.002	0.004 ± 0.000	0.85 ± 0.12456
	82	1.148 ± 0.092	0.106 ± 0.009	0.016 ± 0.000	0.005 ± 0.001	0.9859 ± 0.03998
	92	0.873 ± 0.046	0.082 ± 0.003	0.011 ± 0.000	0.004 ± 0.000	0.8226 ± 0.04954
	102	0.521 ± 0.036	0.053 ± 0.008	0.013 ± 0.001	0.004 ± 0.001	0.7299 ± 0.07419
	112	0.428 ± 0.045	0.066 ± 0.005	0.016 ± 0.001	0.007 ± 0.001	1.3224 ± 0.02609
KG	72	1.447 ± 0.062	0.283 ± 0.015	0.040 ± 0.001	0.008 ± 0.001	5.0302 ± 0.10074
	82	1.307 ± 0.086	0.120 ± 0.003	0.047 ± 0.002	0.004 ± 0.000	2.8502 ± 0.09825
	92	1.255 ± 0.126	0.141 ± 0.025	0.039 ± 0.002	0.005 ± 0.001	2.9682 ± 0.32182
	102	0.836 ± 0.164	0.085 ± 0.015	0.031 ± 0.003	0.004 ± 0.002	1.7054 ± 0.17884
	112	0.738 ± 0.040	0.143 ± 0.012	0.032 ± 0.000	0.006 ± 0.000	3.2061 ± 0.30489
MM	72	7.365 ± 0.527	0.158 ± 0.008	0.032 ± 0.004	0.005 ± 0.001	1.3635 ± 0.22998
	82	6.732 ± 0.307	0.198 ± 0.015	0.030 ± 0.006	0.007 ± 0.001	1.1652 ± 0.12068
	92	5.317 ± 0.178	0.204 ± 0.021	0.028 ± 0.001	0.014 ± 0.004	1.1806 ± 0.04775
	102	5.076 ± 0.387	0.211 ± 0.032	0.027 ± 0.002	0.017 ± 0.005	1.1375 ± 0.12041
	112	4.520 ± 0.330	0.207 ± 0.006	0.030 ± 0.003	0.018 ± 0.001	1.3236 ± 0.06727

[a]5CGA, 5-chlorogenic acid; 4CGA, 4-chlorogenic acid; 3CGA, 3-chlorogenic acid. [b]The data represent the mean of three biological replicates ± standard error. [c]GM, Green Mountain; KG, Keuka Gold; MM, Magic Molly. [d]DAP, days after planting.

Table 2. Amounts of Individual Flavonols[a] during Five Stages of Tuber Development in Three Genotypes[b]

genotype[c]	DAP[d]	Qdg (mg g^{-1})	Krg (mg g^{-1})	Rut (mg g^{-1})	Kmp (mg g^{-1})
GM	72	DL[e]	DL	0.015 ± 0.002	0.043 ± 0.012
	82	DL	DL	0.002 ± 0.0002	0.005 ± 0.002
	92	DL	DL	0.001 ± 0.0002	0.005 ± 0.001
	102	DL	DL	0.004 ± 0.001	0.014 ± 0.005
	112	DL	DL	0.006 ± 0.002	0.013 ± 0.008
KG	72	DL	0.022 ± 0.002	0.022 ± 0.002	0.057 ± 0.025
	82	DL	0.013 ± 0.003	0.013 ± 0.003	0.015 ± 0.006
	92	DL	0.016 ± 0.006	0.016 ± 0.006	0.051 ± 0.023
	102	DL	0.014 ± 0.007	0.014 ± 0.007	0.036 ± 0.004
	112	DL	0.033 ± 0.010	0.014 ± 0.007	0.063 ± 0.008
MM	72	0.120 ± 0.002	DL	0.041 ± 0.004	0.063 ± 0.010
	82	0.115 ± 0.004	DL	0.028 ± 0.004	0.024 ± 0.001
	92	0.079 ± 0.003	DL	0.028 ± 0.002	0.018 ± 0.0005
	102	0.077 ± 0.004	DL	0.025 ± 0.002	0.014 ± 0.001
	112	0.071 ± 0.002	DL	0.026 ± 0.001	0.013 ± 0.001

[a]Qdg, quercetin diglucoside; Krg, kaempferol rutinoside glucoside; Kmp, kaempferol 3-*O*-rutinoside. [b]The data represent the mean ± SE of three biological replicates. [c]GM, Green Mountain; KG, Keuka Gold; MM, Magic Molly. [d]DAP, days after planting. [e]DL, below detection limit.

Two classes of flavonoids (anthocyanins and flavonols) were measured. Tubers have low amounts of flavonols and not all potato genotypes accumulate quantifiable levels of flavonols.[21,33] Quercetin 3-*O* rutinoside (Rut) and kaempferol 3-*O*-rutinoside (Kmp) were present in all genotypes (Table 2). In immature tubers, total flavonols were 2−3-fold higher in MM (0.22 mg g^{-1}) than KG or GM. A marked decrease in flavonols occurred during tuber development in GM (67%) and MM (50%), but not in KG, which showed no clear trend (Figure 3A).

Detectable amounts of anthocyanins were present only in the purple-flesh cultivar, MM (Figure 3B). LC-MS analysis identified 12 individual anthocyanins (Table 3). Total anthocyanins (sum of the 12 individual anthocyanins) were ∼10 mg g^{-1} in immature tubers and decreased by 36% to 6.3 mg g^{-1} in mature tubers (Figure 3), a decrease roughly comparable to a previous result.[10] The two most abundant anthocyanins were petunidin 3-*O*-(6-*p*-coumaroyl)rutinoside-3-glucoside (PtCRG) and peonidin 3-*O*-(6-*p*-coumaroyl)rutinoside-3-glucoside (PeCRG), which contributed 63 and 17%, respectively, of total anthocyanins in tubers of all developmental stages. PtCRG and PeCRG amounts each decreased ∼38% during development. Anthocyanin profiles can be rather specific for each genotype and potentially provide a fingerprint of colored potato cultivars.[34,35]

The purple potatoes had substantially greater amounts of phenylpropanoids than the white and yellow potatoes. Therefore, to evaluate how transcription changed during development and to assess transcript−metabolite relationships, qRT-PCR was used to measure expression of phenylpropanoid genes in MM. The expression of the downstream phenylpropanoid genes, *UFGT* (UDP-glucose:flavonoid 3-*O*-glucosyltransferase), *DFR* (dihydroflavonol reductase), *ANS* (anthocyanin synthase),

Table 3. Anthocyanin Concentrations[a] at Five Stages of Tuber Development in Purple Potatoes (Magic Molly)[b]

DAP	PtCRG (mg g^{-1})	PeCRG (mg g^{-1})	CyCRG (mg g^{-1})	MaCRG (mg g^{-1})	PlCRG (mg g^{-1})	DeCRG (mg g^{-1})
72	6.432 ± 0.098	1.645 ± 0.024	0.519 ± 0.010	0.330 ± 0.009	0.296 ± 0.022	0.248 ± 0.001
82	6.015 ± 0.134	1.551 ± 0.030	0.597 ± 0.015	0.260 ± 0.005	0.248 ± 0.007	0.295 ± 0.007
92	4.858 ± 0.169	1.320 ± 0.023	0.551 ± 0.005	0.213 ± 0.006	0.194 ± 0.007	0.253 ± 0.006
102	4.422 ± 0.328	1.139 ± 0.049	0.553 ± 0.049	0.167 ± 0.005	0.166 ± 0.008	0.294 ± 0.030
112	3.967 ± 0.107	1.030 ± 0.032	0.505 ± 0.033	0.149 ± 0.003	0.151 ± 0.004	0.276 ± 0.016
DAP	PtCaRG (mg g^{-1})	PtCR (mg g^{-1})	DeR (mg g^{-1})	PtR (mg g^{-1})	CyR (mg g^{-1})	PtD (mg g^{-1})
72	0.135 ± 0.007	0.086 ± 0.001	0.062 ± 0.001	0.056 ± 0.001	0.046 ± 0.001	0.037 ± 0.001
82	0.123 ± 0.001	0.081 ± 0.001	0.058 ± 0.002	0.049 ± 0.001	0.043 ± 0.000	0.038 ± 0.001
92	0.106 ± 0.001	0.072 ± 0.001	0.051 ± 0.001	0.043 ± 0.001	0.042 ± 0.001	0.039 ± 0.001
102	0.097 ± 0.001	0.064 ± 0.002	0.049 ± 0.002	0.040 ± 0.0005	0.039 ± 0.001	0.039 ± 0.0005
112	0.089 ± 0.001	0.060 ± 0.001	0.045 ± 0.001	0.036 ± 0.0005	0.035 ± 0.001	0.036 ± 0.0003

[a]PtCRG, PeCRG, CyCRG, MaCRG, PlCRG, and DeCRG are 3-coumaroyl rutinoside 5-glucoside conjugates of petunidin, peonidin, cyanidin, malvidin, pelargonidin, and delphinidin, respectively; PtCaRG, petunidin 3-*O*-(caffeoyl) rutinoside 5-glucoside; PtCR, petunidin 3-*O*-coumaroyl rutinoside, DeR, PtR, and CyR are delphinidin, petunidin, and cyanidin rutinoside, respectively; PtD, petunidin derivative. [b]The data represent the mean ± SE (n = 3).

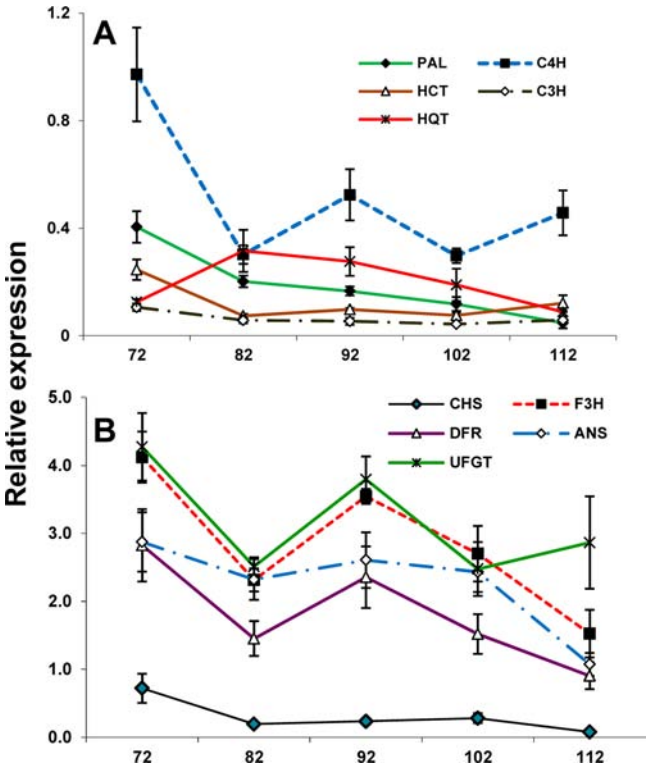

Figure 4. Changes in transcript levels of phenylpropanoid and chlorogenic acid (top) and flavonol and anthocyanin (bottom) biosynthetic genes during five stages of tuber development in Magic Molly. *ANS*, anthocyanin synthase; *F3H*, flavanone 3-hydroxylase; *C3H*, *p*-coumarate 3-hydroxylase; *C4H*, cinnamate 4-hydroxylase; *CHS*, chalcone synthase; *DFR*, dihydroflavonol reductase; *HCT*, hydroxycinnamoyl transferase; *HQT*, hydroxycinnamoyl-CoA quinate transferase; *PAL*, phenylalanine ammonia-lyase; *UFGT*, UDP-glucose:flavonoid 3-*O*-glucosyltransferase. The data represent the mean ± SE (n = 3).

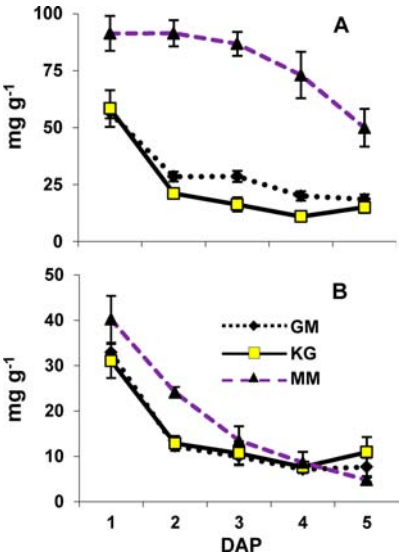

Figure 5. Change in sucrose (A) and glucose (B) content during five stages of tuber development in three genotypes. GM, Green Mountain; KG, Keuka Gold; MM, Magic Molly. The data represent the mean ± SE (n = 3).

and *F3H* (flavanone 3-hydroxylase) was higher than that of the upstream genes, *PAL*, *C4H* (cinnamate 4-hydroxylase), *HQT* (hydroxycinnamoyl-CoA quinate transferase), *C3H* (*p*-coumarate 3-hydroxylase), and *HCT* (hydroxycinnamoyl transferase, Figure 4). Consistent with the decrease in phenolics and anthocyanins observed during tuber development, expression of most genes decreased as tubers matured. For most genes, the decrease in expression was greater between 72 and 82 DAP (~50−75%) than during later stages. The expression of *PAL* and *CHS* in mature tubers was only 10% of that in immature tubers, whereas most other genes were reduced to 30−70%. Of the 10 phenylpropanoid genes examined, only *HQT* expression did not show a clear developmental trend, nor did its expression appear correlated with 5CGA concentrations, which markedly decreased during development. *DFR* is a key gene in anthocyanin biosynthesis, and its expression decreased 68% during development. This differs from an earlier study that reported no change in *DFR* expression in the periderm during development.[20] The decrease in *DFR* expression was greater than the decrease that occurred in anthocyanins, but correlated with anthocyanin concentrations (r = 0.66). These data show that the increase in phenylpropanoids in immature tubers was not limited just to hydroxycinnamic acid derivatives, but extended to other branches of the pathway. Immature tubers from MM had >3-fold higher amounts of anthocyanins than purple potatoes in the previous study,[10] which demonstrates the biosynthetic capacity of potatoes to synthesize sizable amounts of anthocyanins, at least up to 10 mg g^{-1} DW. The amounts of anthocyanins in MM would rank among the upper tier of numerous fruits and vegetables, and a single small serving

dx.doi.org/10.1021/jf401522k | J. Agric. Food Chem. 2013, 61, 7357−7365

Table 4. Carotenoid Amounts during Five Stages of Tuber Development in Three Cultivars[a]

genotype[b]	DAP	violaxanthin (μg g^{-1})	lutein (μg g^{-1})	neoxanthin (μg g^{-1})	antheraxanthin (μg g^{-1})	zeaxanthin (μg g^{-1})	total (μg g^{-1})
GM	72	1.51 ± 0.17	1.27 ± 0.15	0.55 ± 0.03	0.32 ± 0.023	0.036 ± 0.003	4.5 ± 0.37
	82	0.53 ± 0.06	0.48 ± 0.06	0.25 ± 0.03	0.13 ± 0.007	0.019 ± 0.006	1.8 ± 0.17
	92	0.54 ± 0.02	0.54 ± 0.03	0.22 ± 0.01	0.16 ± 0.002	0.015 ± 0.002	1.9 ± 0.08
	102	0.54 ± 0.04	0.52 ± 0.05	0.20 ± 0.01	0.13 ± 0.001	0.019 ± 0.001	1.8 ± 0.08
	112	0.64 ± 0.07	0.69 ± 0.07	0.26 ± 0.02	0.22 ± 0.011	0.036 ± 0.004	2.2 ± 0.19
KG	72	9.24 ± 1.19	6.28 ± 0.97	0.97 ± 0.11	1.51 ± 0.24	0.082 ± 0.004	22.2 ± 3.03
	82	4.54 ± 0.35	2.48 ± 0.26	0.59 ± 0.05	0.80 ± 0.05	0.071 ± 0.009	10.1 ± 0.71
	92	5.59 ± 0.46	2.86 ± 0.23	0.69 ± 0.03	0.95 ± 0.06	0.053 ± 0.005	12.0 ± 0.97
	102	3.84 ± 0.25	1.93 ± 0.11	0.56 ± 0.07	0.68 ± 0.03	0.047 ± 0.007	8.4 ± 0.43
	112	4.64 ± 0.12	2.77 ± 0.13	0.68 ± 0.03	0.99 ± 0.03	0.058 ± 0.002	10.6 ± 0.46
MM	72	0.94 ± 0.07	0.43 ± 0.02	0.21 ± 0.009	0.11 ± 0.004	0.009 ± 0.0005	2.1 ± 0.12
	82	1.35 ± 0.13	0.52 ± 0.04	0.24 ± 0.014	0.18 ± 0.010	0.018 ± 0.002	2.8 ± 0.22
	92	1.31 ± 0.04	0.47 ± 0.03	0.23 ± 0.005	0.20 ± 0.008	0.012 ± 0.002	2.6 ± 0.08
	102	1.29 ± 0.00	0.48 ± 0.02	0.21 ± 0.008	0.16 ± 0.006	0.007 ± 0.0001	2.5 ± 0.02
	112	1.27 ± 0.05	0.51 ± 0.002	0.18 ± 0.013	0.16 ± 0.002	0.008 ± 0.001	2.5 ± 0.07

[a]The data represent the mean ± SE (n = 3). [b]GM, Green Mountain, KG, Keuka Gold; MM, Magic Molly.

would provide many-fold greater amounts than the average intake of 12.5 mg/day/person in the United States.[36]

Sugars. Sucrose is an important regulator of phenylpropanoid biosynthesis in plants;[37] therefore, its role in tubers was assessed, along with that of glucose. Interestingly, immature tubers of the high-phenylpropanoid cultivar, MM, had ∼60% greater sucrose concentrations than GM and KG, whereas MM tubers collected 112 DAP had 168−230% higher amounts (Figure 5). Glucose concentrations were initially higher in MM, but differences disappeared as tubers matured. Consistent with previous results,[32] sugars decreased during development. Thus, sucrose concentrations were highest in the cultivar with highest phenylpropanoid content and also highest in the immature tubers of each cultivar, that is, the developmental stage with the highest phenylpropanoid amounts. These data support a role for sucrose in modulating tuber phenylpropanoid content.

Carotenoids. A previous study of carotenogenesis in greenhouse potatoes reported that carotenoid concentrations were highest in swelling stolon tips and decreased during development in two of three genotypes.[18] The present study differs from the previous in that carotenoid metabolism was measured in field-grown potatoes and used potatoes from later developmental stages, only one of which overlaps with the earlier study. Only KG, a cultivar with light yellow flesh, had appreciable amounts of carotenoids (Table 4). Violaxanthin and lutein were the most abundant carotenoids in KG and decreased substantially between the first and second time points but held steady thereafter (Table 4). A similar trend was seen in GM, which had the second highest amounts of carotenoids, but no developmental effects were observed in MM, the cultivar with the lowest amounts. Thus, like the phenylpropanoids, carotenoids decreased during development, but unlike the phenylpropanoids, carotenoids appeared to decrease only early in the season and then stabilize. Potato cultivars exist that have considerably higher amounts of carotenoids than those used in this study, and it would be interesting to know what happens to their carotenoid profiles during development.

The expression of carotenoid genes during tuber development was studied in KG because of its higher carotenoid amounts. Among the nine carotenoid biosynthetic genes measured, carotene hydroxylase 2 (*CHY2*) was the most abundantly expressed and lutein 1 (*LUT1*) the least (Table 5). During development, the expression of phytoene synthase 1 (*PSY1*) showed no specific trend; although sample to sample variation was considerable, the rest showed a trend to decrease by varying degrees (30−70% of that in immature tubers). Phytoene synthase catalyzes the first committed step in the carotenoid pathway, and *PSY2* transcript levels decreased the most in potatoes harvested 112 DAP. *ZEP* expression decreased during development and was highest in the immature potatoes with higher carotenoid concentrations. Differing results have been reported for *ZEP* expression, with one study finding lower[18] and others reporting higher *ZEP* expression in potatoes with more carotenoids.[29,38] In general, mixed results have been reported about transcriptional control of carotenoid biosynthesis.[38−41]

Glycoalkaloids and Total Protein. Glycoalkaloids have antipest activity and can have health-promoting effects,[42,43] but at higher concentrations they have undesirable side effects, and so new cultivars must contain <20 mg total glycoalkaloids/100 mg fresh weight. Glycoalkaloid concentrations decreased with maturity in all three cultivars (Supporting Information, Supplemental Figure 2), with a greater decrease in GM and MM (62%) than in KG (44%). These data are consistent with previous studies measuring changes in glycoalkaloids at different maturities[32,44,45] and suggest that for cultivars chosen for "baby potato" production, low glycoalkaloid content will be a critical trait and possible bottleneck. Chaconine was present in greater amounts than solanine in the three cultivars, and both glycoalkaloids showed similar decreases during tuber development.

Whereas potatoes do not contain large amounts of protein, the protein is high-quality, among the best from plants, and approaching that of egg.[30,46] Total protein was higher in immature GM tubers (9.6%) than in KG (6.8%) and MM (8.3%, Supporting Information, Supplemental Figure 2). Protein content decreased during the tuber development by 30 and 15% in GM and MM, respectively, but no change was observed in KG samples. In the mature tubers of all genotypes, protein content was similar (∼6.8%, Supporting Information, Supplemental Figure 2). The protein content of potato cultivars

Table 5. Change in Transcript Levels (Relative Expression) of Carotenoid Metabolism Genes[a] during Five Stages of Tuber Development in Keuka Gold[b]

DAP	*PSY1*	*PSY2*	*ZDS*	*LCYe*	*LCYb1*	*LCYb2*	*CHY2*	*ZEP*	*LUT1*
72	0.08 ± 0.01	0.34 ± 0.03	0.03 ± 0.01	0.10 ± 0.03	0.23 ± 0.04	0.05 ± 0.01	3.78 ± 0.63	0.49 ± 0.07	$4.8 \times 10^{-03} \pm 3 \times 10^{-04}$
82	0.18 ± 0.05	0.25 ± 0.06	0.03 ± 0.01	0.07 ± 0.02	0.24 ± 0.07	0.05 ± 0.01	2.69 ± 0.13	0.33 ± 0.13	$3.6 \times 10^{-03} \pm 8 \times 10^{-04}$
92	0.09 ± 0.03	0.28 ± 0.06	0.03 ± 0.00	0.08 ± 0.02	0.13 ± 0.03	0.06 ± 0.01	4.49 ± 1.55	0.34 ± 0.05	$2.3 \times 10^{-03} \pm 4 \times 10^{-04}$
102	0.18 ± 0.06	0.22 ± 0.05	0.02 ± 0.00	0.06 ± 0.01	0.19 ± 0.06	0.04 ± 0.01	2.30 ± 0.56	0.25 ± 0.11	$3.7 \times 10^{-03} \pm 2 \times 10^{-04}$
112	0.02 ± 0.00	0.14 ± 0.03	0.01 ± 0.00	0.04 ± 0.00	0.15 ± 0.03	0.03 ± 0.01	2.08 ± 0.50	0.15 ± 0.02	$1.7 \times 10^{-03} \pm 4 \times 10^{-04}$

[a]*CHY2*, β-carotene hydroxylase 2; *LCYb1* and *2*, lycopene β-cyclase 1 and 2; *LCYe*, lycopene ε-cyclase; *LUT1*, carotene hydroxylase; *PSY1* and *2*, phytoene synthase isoforms; *ZDS*, ζ-carotene desaturase; *ZEP*, zeaxanthin epoxidase. [b]The data represent the mean ± SE (n = 3).

varies, and almost all reported values are for mature potatoes, many of which have higher amounts of protein than the three cultivars used in this study.[30,46] This work suggests immature potatoes from high-protein cultivars may be likely to have even higher amounts than reported at maturity, which is a point that awaits additional research.

Complex mechanisms will influence a tuber's phytonutrient content. Phenylpropanoid metabolism is influenced by the environment,[47] and thus the absolute amount of these compounds found in any cultivar is not likely to be a constant, but will vary within a certain range. In Alaska, the climate for potato growth can be described as high-light, extraordinarily long photoperiods, with relatively cool soil temperatures.[48] The bulking phase of tuber growth may have a dilutive effect on metabolites that accounts for some of the decrease during development, but other factors are also at play, such as increased expression of some phytonutrient genes in immature potatoes.

In summary, phenolic acids, flavonols, anthocyanins, carotenoids, and protein were present in greater concentrations in immature potatoes harvested from young plants than in mature tubers. "Baby" or "new" potatoes are widely available, and these results suggest they contain higher amounts of a broad range of dietarily desirable compounds. Consequently, baby potatoes may especially appeal to health-conscious consumers and present a palatable, inexpensive opportunity to increase dietary intake of these phytonutrients. A more extensive survey of baby potato germplasm is warranted to identify cultivars with the highest amounts of phytonutrients, high tuber set, and low amounts of glycoalkaloids.

■ ASSOCIATED CONTENT

Supporting Information

LC-MS data used to identify compounds (Supplemental Table 1); quantitation of various hydroxycinnamic acid derivatives (Supplemental Table 2); quantitation of three aromatic amino acids (Supplemental Table 3); tuber weights of samples collected 10 days apart (Supplemental Figure 1); changes in protein and glycoalkaloid content during development (Supplemental Figure 2). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ACKNOWLEDGMENTS

We thank Syam Kumar for technical support and are grateful to the Washington State Potato Commission for their support.

■ ABBREVIATIONS USED

3,4,5CGA, *cis*-4CGA and *cis*-5CGA, chlorogenic acid isoforms; *ANS*, anthocyanin synthase; BDCS, bis(dihydrocaffeoylspermine); BDCSD, bis(dihydrocaffeoylspermidine); *C3H*, *p*-coumarate 3-hydroxylase; *C4H*, cinnamate 4-hydroxylase; CA, caffeic acid; *CHS*, chalcone synthase; *CHY2*, β-carotene hydroxylase 2; CyCRG, cyanidin 3-*O*-(6-*p*-coumaroyl)rutinoside-5-glucoside; CyR, cyanidin rutinoside; DeCRG, delphinidin 3-*O*-(6-*p*-coumaroyl)rutinoside 5-glucoside; DeR, delphinidin rutinoside; *DFR*, dihydroflavonol reductase; *F3H*, flavanone 3-hydroxylase; FRAP, ferric reducing ability of plasma; GM, Green Mountain; *HCT*, hydroxycinnamoyl transferase; *HQT*, hydroxycinnamoyl-CoA quinate transferase; KG, Keuka Gold; Kmp, kaempferol 3-*O*-rutinoside; Krg, kaempferol rutinoside glucoside; *LCYb1* and *2*, lycopene β-cyclase 1 and 2; *LCYe*, lycopene ε-cyclase; *LUT1*, carotene hydroxylase; MaCRG, malvidin 3-coumaroyl-rutinoside 5-glucoside; MM, Magic Molly; *PAL*, phenylalanine ammonia-lyase; PeCRG, peonidin 3-*O*-(6-*p*-coumaroyl)-rutinoside-5-glucoside; PlCRG, pelargonidin 3-*O*-(6-*p*-coumaroyl)rutinoside-5-glucoside; *PSY1* and *2*, phytoene synthase isoforms; PtCaRG, petunidin 3-*O*-(6-*p*-caffeoyl)-rutinoside-5-glucoside; PtCR, petunidin 3-*O*-(6-*p*-coumaroyl)-rutinoside; PtCRG, petunidin 3-*O*-(6-*p*-coumaroyl)rutinoside-5-glucoside; PtD, petunidin derivative; PtR, petunidin rutinoside; Qdg, quercetin diglucoside; Rut, quercetin-3-rutinoside; TDCS, tris(dihydrocaffeoylspermine); TDCSD, tris-(dihydrocaffeoylspermidine); *UFGT*, UDP-glucose:flavonoid 3-*O*-glucosyltransferase; *ZDS*, ζ-carotene desaturase; *ZEP*, zeaxanthin epoxidase

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