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Betalains in Red and Yellow Varieties of the Andean Tuber Crop Ulluco (*Ullucus tuberosus*)

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The betalain pigments in ulluco (*Ullucus tuberosus*), a tuberous crop native to the Andes, have been investigated for the first time using LC−DAD−ESI-MS−MS^2 analyses. Five red, yellow, and red-spotted accessions introduced into New Zealand as a new food crop plus two red tetraploid lines were investigated. Thirty-two different betalains were identified. Both the yellow and red tubers were rich in yellow betaxanthins, and the most prominent among the 20 identified were histidine-betaxanthin, arginine-betaxanthin and glutamine-betaxanthin. Arginine-betaxanthin has been reported to occur naturally only once before and was found in yellow ulluco but not in the red tubers. Twelve betacyanins were found in red tubers, with roughly 50% of this content being betanin/isobetanin. Betacyanin levels were up to 70 μg/g fresh weight in red tubers, but were below quantifiable levels in yellow tubers. Betaxanthin levels were up to 50 μg/g fresh weight in yellow tubers. Interference by betacyanins in measuring levels of betaxanthins by visible spectrophotometry is discussed. Low concentrations of betalains were detected in leaves, whereas stems contained total levels similar to the tubers, with dopamine-betaxanthin and betanin being the major pigments. This is the first report describing both the betacyanin and betaxanthin patterns in a plant from the Basellaceae family.

KEYWORDS: Ulluco; Ullucus tuberosus; Basellaceae; betalains; betacyanins; betaxanthins

INTRODUCTION

Ulluco (*Ullucus tuberosus* Caldas, family Basellaceae) is an important Andean crop plant (*1*) commonly grown at altitudes of 3000−3800 m (*2*). Ulluco is one of the root and tuber crops which today are common staples for an estimated 25 million people in the Andean highlands (*2*), and the leaves are also eaten (*3*). Tubers can be stored for up to a year, and the crispness of the uncooked tuber is maintained after cooking (*1*). This crop has so far had only a small impact outside South America, with introduction attempts in Europe largely unsuccessful (*2, 4*). Nevertheless, it is a crop worth developing further, one reason being the presence of betalain pigments. In addition to being colorful, some betalains have antioxidant, anti-inflammatory, antimalarial, and antitumoral activities (*5–11*). It has also been suggested that a regular intake of betalain-containing foods can provide protection against stress-related conditions (*5*). There are currently few sources of betalains in the average diet, so alternative vegetable or fruit sources are desirable. Ulluco is now being grown commercially in New Zealand after successful introduction at latitudes similar to the Andes but at low altitude (*3*).

Ulluco tubers can be white, yellow, pink, orange, magenta, or red or with red spots on a yellow background (*2*). The ulluco accessions established in New Zealand gave red, red-spotted, and yellow tubers (**Figure 1**), and consumer panelists preferred the red tubers (*3*). The pigment composition of the available accessions was examined as part of a breeding program for selection of ulluco cultivars with improved consumer acceptability. Ulluco has recently been identified as a betalain-containing crop by Campos et al. (*12*), who found yellow betaxanthins at levels ranging from 22 to 96 μg/g of fresh weight in 15 genotypes and red betacyanins at 64 μg/g in one genotype. However, there are no reports identifying the individual compounds responsible for the rich colors of ulluco, which are potentially also responsible for the antioxidant properties of this crop (*12*).

Betalains are pigments found in some higher fungi and in a few plant families of the order Caryophyllales, which includes the ulluco family Basellaceae (*13, 14*). Only one report was found identifying individual betalains from any of the Basellaceae genera: the fruits of *Basella rubra* contained gomphrenin I (15(*S*)-betanidin 6-*O*-β-glucoside, **Figure 2**) as the major

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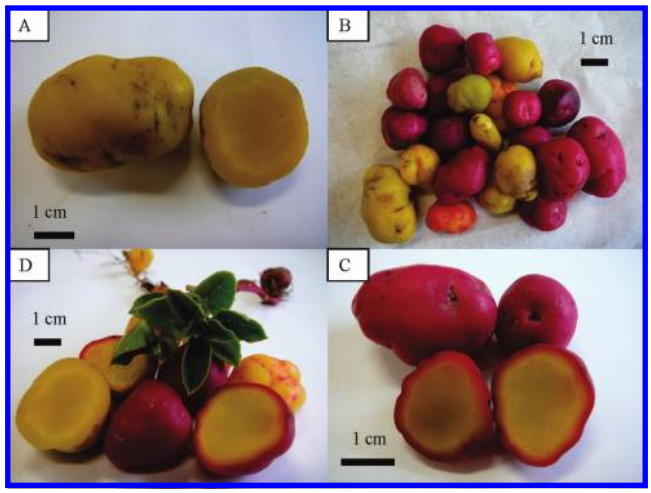

Figure 1. Examples of ulluco tubers used in this study: **A**, yellow accession U9; **B**, mixture of differently colored tubers; **C**, red ulluco showing the localization of the betacyanins in the surface layer; **D**, accessions U9, U13 (spotted), and red U15 together with juvenile plants of U13 and U15.

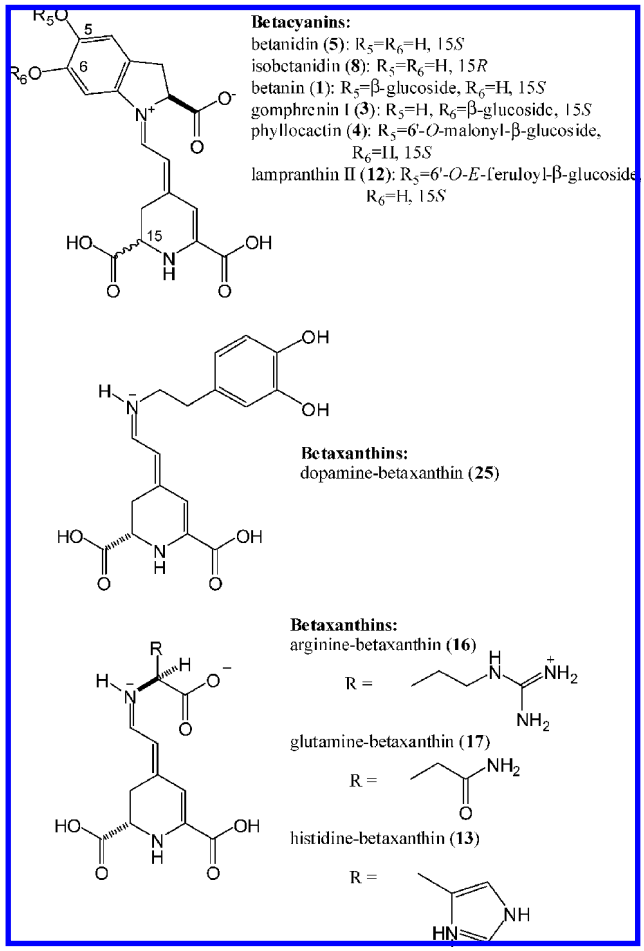

Figure 2. Structures of major betalains identified in ulluco tubers.

betacyanin plus lower levels of 4-coumaroyl and feruloyl derivatives and 15(*R*) isomers (*15*). We could not find any reports identifying specific betaxanthins from the Basellaceae family.

The betalains are divided into the yellow betaxanthins, which are imino condensation products between betalamic acid and amines (especially amino acids), and the red/purple betacyanins formed by condensing betalamic acid and *cyclo*-Dopa (**Figure 2**) (*13*). The betacyanins vary by *O*-glycosylation with a range of glycosides in either the 5- or 6-position of the *cyclo*-Dopa moiety, plus further acylation of the glycoside part with ferulic, cinnamic, or malonic acids. Betalains are interesting natural food coloring agents due to their range of colors plus stability between pH 3 and 7 (*16, 17*). Betanin (betanidin 5-*O*-β-glucoside, **Figure 2**) from the deep-red beetroot (*Beta vulgaris*) has historically been the most used compound, but recent discoveries of edible sources such as cactus fruits, a range of Amaranthaceae plants, pitaya, yellow beet, and Swiss chard have provided alternative sources (*18–24*). The extensive research by the Stintzing and Corke groups have revealed the betalain composition of a range of commercially important colored crops; for example, yellow beet, *Amaranthus*, purple pitaya, and cactus pear, and the list of identified betalains has reached 55 (*16*). We now report the identification of the individual type of betaxanthins and betacyanins in ulluco and their levels in seven red and yellow accessions.

MATERIALS AND METHODS

Plant Material. The seven ulluco accessions used in this study were the red (U2 and U15), red-spotted on yellow (U13), and yellow (U3 and U9) accessions introduced to New Zealand from South America as described by Busch et al. (*3*) plus tetraploids (U4n3 and U4n4) of U2 prepared by treating rapidly growing and proliferating in vitro plant tissue with oryzalin (15.5 mg/L). These were all grown at the Crop & Food Research farm at Lincoln, New Zealand (43° 39′ S, 172° 29′ E, 11 m above sea level) and were gathered 20 weeks after sowing, in May 2007. The tubers were stored in the dark at 3 °C at ambient humidity (conditions previously shown to preserve quality (*3*)) until analyzed in December 2007. Leaves and stems from accessions U13 and U15 grown at Invermay Research Centre (45° 51′ S, 170° 23′ E, 50 m above sea level) were harvested in December 2007 and stored similarly. Purple beetroot, purple-pink flowers of *Gomphrena globosa* L., and purple stems of Swiss chard (*Beta vulgaris* L. ssp. *cicla* [L.] Alef. cv. 'Bright Lights'), used for generation of reference compounds, were obtained from local New Zealand sources.

Pigment Extraction. A modified version of the method of Kugler et al. (*24*) was used, with two or three individual tubers from different plants from each accession extracted in parallel. Tubers were washed then finely chopped by hand and frozen in liquid nitrogen. Each tuber (5−15 g, accurately weighed) was added to 60% aqueous methanol solution containing 50 mM sodium ascorbate, with 5 mL solvent per 1 g of tuber, and the slurry was homogenized for 60 s using an T25 Ultraturrax (IKA-Labortechnik, Staufen, Germany). The slurry was further stirred at room temperature for 40 min before the solid plant material was removed via suction filtration through a No 131 filter paper (Toyo, Tokyo, Japan). Methanol was removed from the extract by rotary evaporation at 30 °C, then the concentrate was freeze-dried overnight. Resuspension in ~15 mL (accurately measured) of deionized water then filtration (0.45 μm Acrodisc) gave a clear solution, which was stored at −25 °C until analysis. The ulluco stems and leaves and other plant materials were treated in an identical manner.

LC−MS Analyses. The LC−MS system consisted of a Thermo Electron Corporation (San Jose, CA) Finnigan Surveyor MS pump, Finnigan MicroAS autosampler, Finnigan Surveyor PDA detector, and a ThermaSphere TS-130 column heater (Phenomenex, Torrance, CA). Extract subsamples (1 mL) were dried down then dissolved in 1 mL of aqueous methanol (1:1 v/v), centrifuged, and filtered (0.22 μm nylon) into amber glass vials and placed in an autosampler rack maintained at 10 °C. A 5 μL aliquot of each prepared extract was separated by reversed-phase chromatography employing a 4 mm × 2 mm i.d., 10 μm, Aqua guard cartridge and a 250 mm × 2.1 mm i.d., 4 μm, Synergi-HydroRP C_{18} column (Phenomenex, Torrance, CA) maintained at 25 °C using the two different eluent systems and gradients described below for the analytical LC analyses. Extracts of red (U2R2) and yellow (U9R1) ulluco tubers were analyzed, plus beetroot and *G. globosa* extracts for reference purposes. The eluent was scanned by DAD from 210−600 nm and analyzed by API-MS (LTQ, 2D linear ion-trap,

Thermo-Finnigan, San Jose, CA) with electrospray ionization (ESI) in the positive ion mode. Data were acquired for parent masses *m/z* 145−1500, which were selected for MS^2 fragmentation. Parent ions were excluded for 15 s after daughter ion fragmentation to allow data collection of coeluting parent or low-intensity ions.

LC−DAD Analyses. Pigments were separated on an Agilent 1100 system fitted with a G1315B diode array detector. The column employed was a 250 mm × 4.6 mm i.d., 5 μm, Luna C18(2) analytical C_{18} reversed-phase column (Phenomenex) fitted with a 4 mm × 2 mm i.d. C_{18} reversed-phase guard column. Samples (50 mL) were analyzed at 25 °C at a flow rate of 1 mL/min. Two different elution strategies were employed using 1% formic acid in water (v/v, eluent A) and a mixture of MeCN in water of 80:20 (v/v, eluent B) as previously described (*24*). Betaxanthins were separated starting isocratically with 100% A for 2 min, followed by a linear gradient from 0 to 20% B in 60 min and a subsequent linear gradient from 20 to 100% B in 5 min. Separation of betacyanins was accomplished beginning with 2% B in A at 0 min, followed by a linear gradient to 33% B in A in 30 min. Betaxanthins and betacyanins were monitored and quantified at 470 and 538 nm, respectively. Peak areas were standardized for extraction volume and tuber fresh weight to give the data in **Table 1**. Reproducible column performance during the investigation was ascertained by analysis of multiple injections of an ulluco extract (U9) throughout the study. The betacyanin isomerization over time was studied by repeated injections of a purple Swiss chard extract over 48 h.

Spectrophotometric Analyses. The concentrations of betaxanthins and betacyanins in the extracts were determined using a JASCO V-550 UV/vis spectrophotometer and a modified version of the method described by Kugler et al. (*25*): 200 μL aliquots of extract were diluted with 1800 μL of McIllvaine buffer, pH 6.0, after which the absorbance was recorded between 300 and 700 nm using McIllvaine buffer as the blank. The absorbance at maxima, at 470−480 nm for betaxanthins and at 538 nm for betacyanins, were used to calculate the concentration of the pigments [B] in milligrams per gram of fresh tuber according to the formula:

$$[\mathrm{B}] = \frac{(A\mathrm{Df}V\mathrm{Mw})}{(mL\varepsilon)}$$

where A = absorption at the absorption maximum, Df = dilution factor (10 in this case), V = final volume (mL) of each extract, Mw = molar weight of the most abundant betacyanin (betanin, 550 g/mol) and betaxanthin (histidine-betaxanthin, 348 g/mol), m = mass of the tuberous material (g) used to generate the analyzed extract, L = cuvette length (1 cm), and ε = extinction coefficient. For betacyanin determination, the extinction coefficient for betanin (60 000 L/mol × cm) was used, whereas a mean extinction coefficient of 48 000 L/mol × cm was used for approximating the amount of betaxanthins (*24, 26*). Each tuber extract was analyzed in triplicate, showing relative standard deviations about ±2%. Average values are given in **Table 1**.

RESULTS AND DISCUSSION

Identification of Betalains. Identification of already reported betalains was achieved using LC−DAD−ESI-MS−MS^2, and comparisons with readily available beetroot, purple Swiss chard, and *G. globosa* flowers, whose pigment compositions have already been reported (*23–25*). This approach led to the identification of 12 betacyanins and 20 betaxanthins in our ulluco samples.

The main betacyanins in a red ulluco tuber extract (**Figure 3**) were isomers, with $[M + H]^+$ at *m/z* 551 and loss of glucose to give a betanidin fragment at *m/z* 389 in MS^2 (**Table 2**). These peaks were identified as betanin and isobetanin, with the further isomers gomphrenin I and isogomphrenin I eluting later (**Figure 2**), on the basis of reported relative retention times (*24, 25*). Betanidin and isobetanidin were readily distinguished by having the lowest *m/z* molecular ions, which showed losses of both CO_2 and formic acid in MS^2 (**Table 2**). Phyllocactin and isophyllocactin showed losses of CO_2 and malonyl groups in

Table 1. Levels of Betacyanins (Bc) and Major Betaxanthins (Bx) in the Individual Tuber Samples

		LC peak areas[a]													vis spectrophotometry	
ulluco sample	tuber color	betanin	isobetanin	phyllocactin	betanidin	isophyllocactin	isobetanidin	BMG[b]	IBMG[c]	LP II[d]	ILP II[e]	total Bc	His-Bx	Arg/Gln-Bx	Glx-Bx	total Bx	Bx Vis (μg/g)	Bc Vis (μg/g)
U2R2	red	2.64	0.53	0.24	0.97	0.14	0.09	0.43	0.10	0.69	0.16	5.98	0.75	0.20	0.17	1.12	29.6	54.2
U2R3	red	2.10	0.45	0.18	0.86	0.20	0.09	0.46	0.12	0.74	0.18	5.38	1.34	0.56	0.26	2.16	34.3	52.8
U3R1	yellow	0.02	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	0.02	2.02	3.27	1.18	6.47	51.0	ND[f]
U3R3	yellow	0.04	0.01	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	0.05	2.22	0.48	0.82	3.51	36.0	ND[f]
U3R4	yellow	0.03	0.01	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	0.05	1.85	1.74	1.51	5.11	40.4	ND[f]
U4n3R1	red	5.24	1.20	0.43	0.79	0.13	0.07	0.37	0.10	0.90	0.22	9.46	1.57	1.80	0.41	3.77	42.0	71.1
U4n3R2	red	4.22	0.78	0.41	0.89	0.16	0.06	0.35	0.08	0.80	0.18	7.91	0.23	0.07	0.16	0.46	29.3	70.3
U4n3R4	red	4.08	0.88	0.40	1.32	0.27	0.10	0.28	0.08	0.67	0.16	8.23	0.47	0.07	0.07	0.61	24.3	69.8
U4n4R1	red	3.20	0.86	0.42	1.29	0.23	0.15	0.24	0.08	1.40	0.37	8.25	2.76	2.71	0.62	6.09	55.1	66.1
U4n4R2	red	4.22	0.84	0.23	1.10	0.14	0.08	0.31	0.08	0.61	0.16	7.78	1.21	1.12	0.35	2.69	36.7	61.7
U9R1	yellow	0.02	0.01	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	0.03	1.92	3.55	0.85	6.32	45.6	ND[f]
U9R4	yellow	0.03	0.03	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	0.05	2.50	2.98	0.96	6.44	53.6	ND[f]
U13R2	spotted[g]	0.11	0.05	0.02	0.08	0.02	0.01	0.06	0.03	0.10	0.02	0.49	3.23	7.43	1.54	12.20	72.6	5.6
U13R3	spotted[g]	0.14	0.05	0.05	0.08	0.02	ND	0.04	0.02	0.12	0.03	0.55	1.67	0.77	0.39	2.83	28.4	5.6
U13 stem	red/brown	1.14	0.14	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	1.28	0.08	ND[f]	0.13	0.21	20.9	15.6
U13 leaves	green	0.17	0.04	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	0.21	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	3.5
U15R1	red	1.83	0.28	0.16	0.63	0.05	0.06	0.59	0.11	0.80	0.19	4.72	1.80	1.54	0.81	4.15	39.4	42.3
U15R3	red	1.39	0.27	0.09	0.65	0.08	0.08	0.41	0.09	0.91	0.25	4.24	1.10	0.21	0.29	1.60	27.8	37.9
U15R4	red	1.94	0.29	0.11	0.67	0.07	0.06	0.50	0.10	0.80	0.19	4.74	0.96	0.38	0.42	1.77	28.6	43.4
U15 stem	red	8.74	0.96	0.03	0.73	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	10.46	0.06	ND[f]	0.03	0.09	36.5	86.7
U15 leaves	red/green	0.49	0.11	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	0.60	ND[f]	ND[f]	ND[f]	ND[f]	ND[f]	5.6

[a] Arbitrary units, standardized for extraction volume and tuber weight. [b] Betanidin-monoferuloyl-5-*O*-β-diglucoside. [c] Isobetanidin-monoferuloyl-5-*O*-β-diglucoside. [d] Lampranthin II. [e] Isolampranthin II. [f] Not determined. [g] Yellow tuber with red spots.

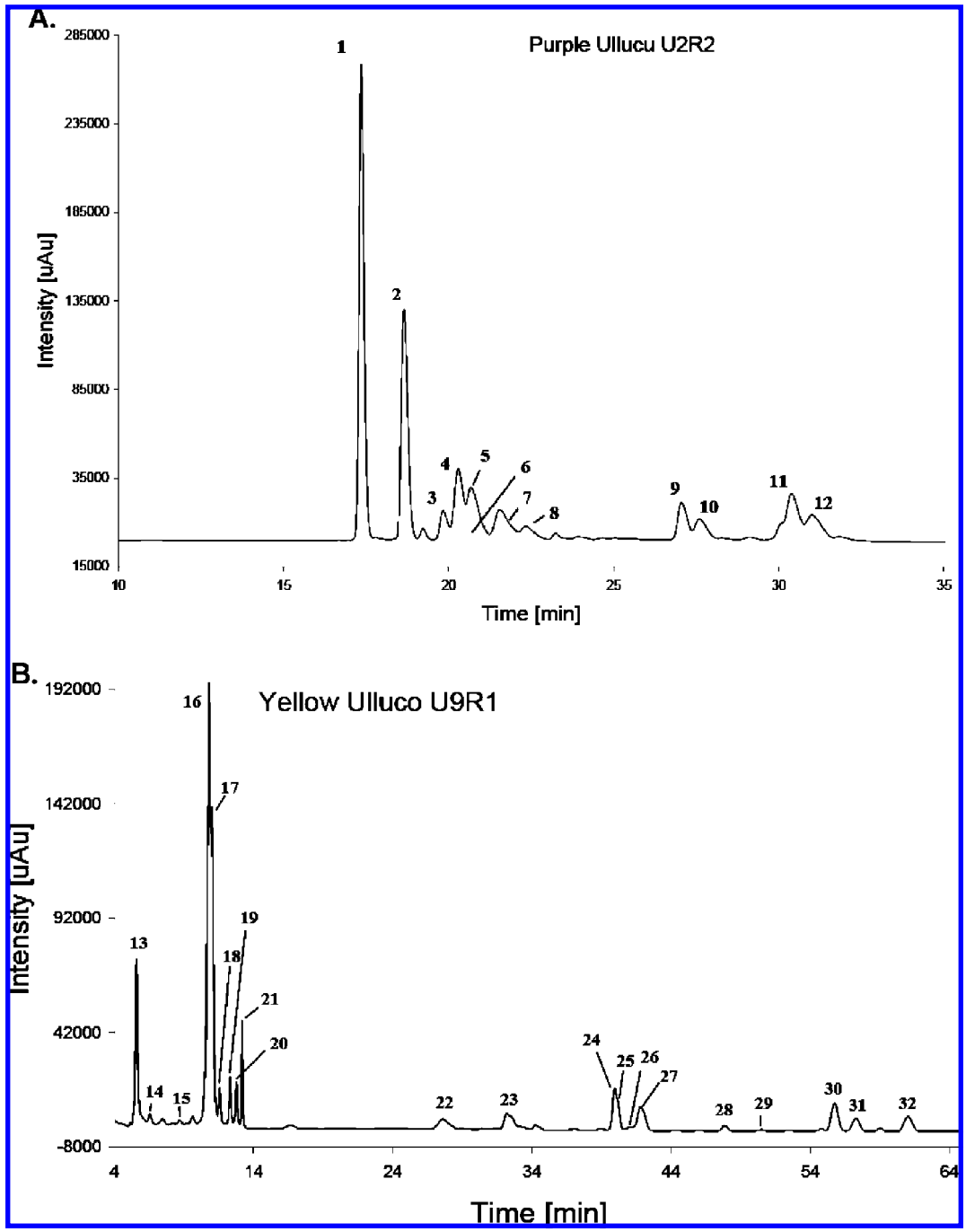

Figure 3. LC trace of betacyanins in red ulluco U2R2 detected at 538 nm (**A**) and betaxanthins in yellow ulluco U9R1 detected at 470 nm (**B**). The peak assignments are in **Table 2**. Refer to **Figure** S1 for close-ups of the chromatogram for tuber U9R1.

MS^2, as well as the main betanidin fragment (**Table 2**). The betacyanin pair eluting at 27.1 and 27.7 min were tentatively identified as betanidin−monoferuloyl-5-*O*-*β*-diglucoside/isobetanidin−monoferuloyl-5-*O*-*β*-diglucoside on account of their relative retentions and comparison with a reference extract from purple Swiss chard (*24*). Other compounds eluted with these two compounds, and it was not possible to obtain clean MS data. The most retained betacyanins were identified as lampranthin II and isolampranthin II. This pair could be distinguished from isomeric betacyanins ($[M + H]^+$ at *m*/*z* 727, and the same 389 and 551 fragments in MS^2), such as amaranthin/isoamaranthin and a range of gomphrenin isomers, by their retention times and λ_{max} values. Amaranthin/isoamaranthin are more polar and elute before betanidin, whereas the less polar gomphrenin isomers have a λ_{max} of 544 nm (λ_{max} II) (*25, 27*). This gave a total of 12 betacyanins identified in ulluco tubers (**Table 2**).

Identification of betaxanthins by LC−DAD−ESI-MS−MS^2 analyses was more straightforward than for the betacyanins, since fewer isomers were present (**Table 2**). The isomeric pairs glutamine-betaxanthin/lysine-betaxanthin and isoleucine-betaxanthin/leucine-betaxanthin were assigned on the basis of relative retention times (*24, 25*). We note for future reference that the main MS^2 peak for glutamine-betaxanthin was at *m*/*z* 323, as compared to *m*/*z* 296 for MS^2 of lysine-betaxanthin (**Table 2**). Arginine-betaxanthin, coeluting with glutamine-betaxanthin, was detected at high levels in a yellow ulluco tuber extract (**Figure 3**) but was not detected in a red-skinned tuber extract (**Table**

Table 2. Betalains Identified in Ulluco with HPLC−DAD−ESI-MS−MS^2 Data

	t_R min	λ_{max} nm	$[M + H]^+$ *m/z*	MS^2 *m/z*
betacyanin				
betanin (**1**)	17.5	534	551	389
isobetanin (**2**)	18.6	534	551	389
gomphrenin I (**3**)	19.8	534	551	389
phyllocactin (**4**)	20.4	534	637	389
betanidin (**5**)	20.7	540	389	343
isogomphrenin I (**6**)	20.8	ND[a]	551	389
isophyllocactin (**7**)	21.5	534	637	389
isobetanidin (**8**)	22.4	540	389	343
betanidin-monoferuloyl-5-*O*-β-diglucoside (**9**)[b]	27.1	534	889[c]	ND[a]
isobetanidin-monoferuloyl-5-*O*-β-diglucoside (**10**)[b]	27.7	534	889[c]	ND[a]
lampranthin II (**11**)	30.4	534	727	389
isolampranthin II (**12**)	31.0	534	727	389
betaxanthin				
histidine-betaxanthin (**13**)	5.6	472	349	305
asparagine-betaxanthin (**14**)	7.3	469	326	ND[d]
serine-betaxanthin (**15**)	8.8	468	299	ND[d]
arginine-betaxanthin[e] (**16**)	10.8	469	386	324
glutamine-betaxanthin (**17**)	11.0	470	340	323
aspartic acid-betaxanthin (**18**)	11.6	469	327	309
lysine-betaxanthin (**19**)	12.3	458	340	296
threonine-betaxanthin (**20**)	12.8	469	313	269
glutamic acid-betaxanthin (**21**)	13.2	469	341	297
proline-betaxanthin (**22**)	27.6	479	309	291
dopa-betaxanthin (**23**)	32.2	472	391	347
tyrosine-betaxanthin (**24**)	39.9	471	375	ND[d]
dopamine-betaxanthin (**25**)	40.0	459	347	303
methionine-betaxanthin (**26**)	41.1	470	343	325
valine-betaxanthin (**27**)	41.8	469	311	267
tyramine-betaxanthin[e] (**28**)	47.8	460	331	287
3-methoxytyramine-betaxanthin[e] (**29**)	50.1	462	361	ND[d]
isoleucin-betaxanthin (**30**)	55.7	470	325	281
leucin-betaxanthin (**31**)	57.4	469	325	281
phenylalanine-betaxanthin(**32**)	61.0	472	359	315

[a] Not determined due co-eluting compounds. [b] Tentatively identified only due to coeluting compounds. [c] One of many masses detected. [d] Not determined, compound at low concentration. [e] Found by LC−MS in yellow tuber (U9R1), not in red-skinned tuber (U2R2).

2). This is only the second instance of arginine-betaxanthin occurring naturally, following the recent report of its presence in red petals of *G. globosa* (*25*). A total of 20 betaxanthins were identified in ulluco tubers (**Table 2**).

LC Analyses of Betalain Levels in Ulluco Accessions. We analyzed two or three individual tubers from each of red (U2 and U15), red-spotted on yellow (U13), and yellow (U3 and U9) accessions, plus tetraploids (U4n3 and U4n4) of U2 prepared by chemical treatment, plus leaves and stems from accessions U13 and U15. Extracts of these 21 samples were analyzed by two different LC methods separately optimized for betacyanins and betaxanthins as previously described (*24*). However, the betacyanin LC method gave overlapping peaks for phyllocactin (**4**), betanidin (**5**), and isogomphrenin I (**6**) (**Figure 3**), so accurate quantification of the gomphrenin I pair of isomers, the least abundant betacyanins, was not possible. Repeat analyses of one ulluco extract at intervals during the analyses showed that the betanin peak became smaller with time (by ~25% over 48 h at room temperature, **Figure** S2), and the isobetanin peak grew correspondingly. This isomerization of betacyanins at the chiral center of the betalamic acid unit moiety (C_{15}, **Figure 2**) has been noted previously (*13*). Therefore, we report betacyanin levels as the sum of isomeric pairs, since this remained reasonably constant during the time taken for workup and analysis. The LC conditions optimized for betaxanthin separation previously reported by Stintzing et al. (*24, 28*) gave overlapping peaks for some of the major betaxanthins: glutamine-betaxanthin with arginine-betaxanthin and tyrosine-betaxanthin with dopamine-betaxanthin (**Table 2**). Peak areas were standardized for extraction volume and tuber fresh weight.

The most striking feature of the LC data for the different ulluco accessions is, as expected, the difference in total betacyanin levels between the red-skinned and all yellow tubers (**Figure 4**). Although the LC method was not calibrated with response factors for the betacyanins, the total peak area does correlate well with the conventional spectrophotometric method for quantifying these compounds. Elevated levels of betacyanins, as compared with the other red-skinned accessions, were detected in the tetraploids U4n3 and U4n4, whereas the red-spotted U13 tubers contained much lower amounts of betacyanins, as expected. Betacyanins were found in the stems at higher concentrations than in tubers of the same accession (**Figure 4**), but the leaves contained only low levels of betacyanins, presumably in the red veins.

The relative levels of the different betacyanins in the red-skinned and red-spotted ulluco tubers were all similar, with betanin/isobetanin the major components in all the samples (**Table 3**). Only the betanins were detected at very low levels in the yellow accessions. Therefore, the betacyanin profile of ulluco tubers is similar to that of purple Swiss chard (*24*), except that ulluco tubers contain low levels of gomphrenin I/isogomphrenin I and generally lower proportions of betanin/isobetanin. The variety of betacyanins found in the tubers was not present in the stems and leaves analyzed because >90% of the betacyanins were betanin and isobetanin. The higher level of

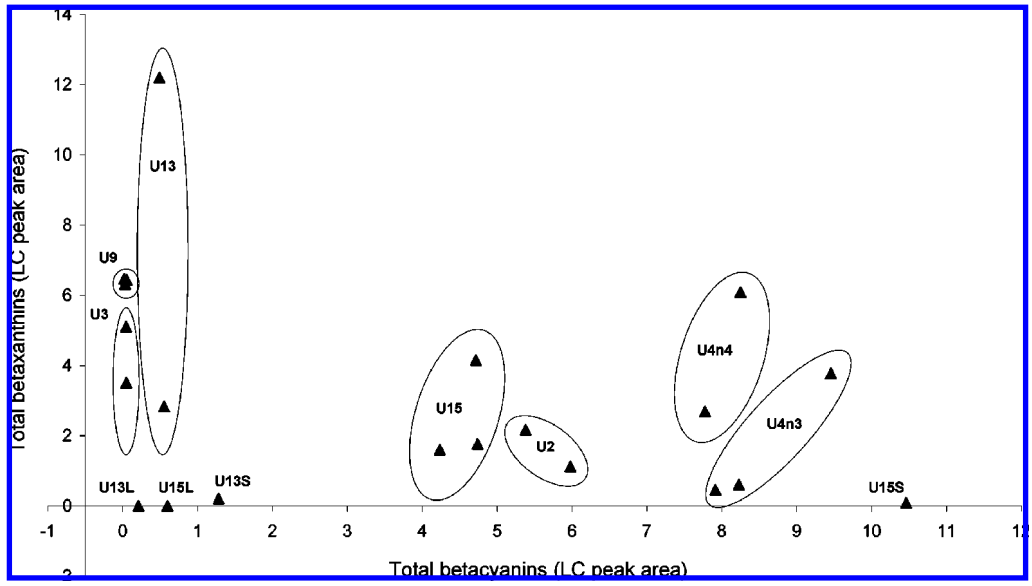

Figure 4. Total betacyanin and betaxanthin levels (based on standardized peak areas) of ulluco tubers from different accessions, plus stems (S) and leaves (L).

Table 3. Betacyanin Proportions in Red-Skinned Ulluco Accessions

	relative levels (% of total betacyanin LC peak area)[a]				
betacyanin pair	U2 (2)[b]	U4n3 (3)	U4n4 (2)	U13 (2)	U15 (3)
betanins	50.2 ± 3.9	63.9 ± 4.5	57.3 ± 11.5	32.9 ± 3.2	43.5 ± 4.5
phyllocactins[c]	6.7 ± 1.5	7.0 ± 1.2	6.2 ± 2.2	10.4 ± 3.8	4.2 ± 1.1
betanidins[c]	17.6 ± 0.3	12.8 ± 4.1	16.3 ± 1.8	16.4 ± 1.4	16.1 ± 1.8
BMGs[d]	9.8 ± 1.4	4.9 ± 0.6	4.5 ± 0.8	14.9 ± 6.4	13.1 ± 1.6
lampranthin IIs	15.6 ± 2.1	11.4 ± 1.2	15.8 ± 8.2	25.4 ± 1.2	23.1 ± 3.6

[a] Mean ± standard deviation. [b] Number of tubers analyzed. [c] Values have been calculated, even though there is some overlap of peaks. [d] Betanidin-monoferuloyl-5-O-β-diglucoside and its isomer.

betacyanins in the tetraploid lines U4n3 and U4n4 (**Figure 4**) shows the potential for breeding ulluco with higher pigment levels, as has been achieved for *B. vulgaris* (*13*).

The major betaxanthins in all of the ulluco tubers analyzed were histidine-betaxanthin, glutamine-betaxanthin coeluting with arginine-betaxanthin, and glutamic acid-betaxanthin. Since the other betaxanthins were generally detected in much lower amounts, the total areas of these three LC peaks were used as a measure of total betaxanthin content. Total betaxanthin contents varied much more between tubers of the same ulluco accession than did the betacyanin content (**Table 1** and **Figure 4**). Furthermore, the relative levels of these major betaxanthins varied much more than did the relative levels of the betacyanins: for example, the glutamine-betaxanthin/arginine-betaxanthin peak was 50% of the total betaxanthins in one U3 accession tuber, but 14% in another. We cannot find any other reports on the variability of betaxanthin (or betacyanin) proportions within different samples of the same parts of a single plant species. This variability in absolute and relative contents of betaxanthins is probably a consequence of the decisive step in betaxanthin biosynthesis being a spontaneous reaction (*29*), rather than being under enzymatic control. Therefore, the levels and proportions of betaxanthins will depend on the levels and proportions of amino acids available to react with betalamic acid in each tuber. The different stages of maturation and soil depths might affect betaxanthin distribution for the different tubers analyzed, but these factors were not studied. Much lower levels of betaxanthins were found in the ulluco stems as compared to tubers, and none were detected in the leaves, so these yellow pigments are mostly located in the underground tubers. The relative betaxanthin content of ulluco stems differed from the tubers, with dopamine-betaxanthin being the most prevalent betaxanthin, perhaps reflecting high dopamine levels in stems (*29*).

Betalain Contents by Visible Spectrophotometry. The LC analyses described above were time-consuming because of the long solvent-gradient programs and the two separate analyses of betacyanins and betaxanthins, and they were not calibrated with response factors for each compound to provide absolute levels. In all the published reports that we are aware of, the total levels of betalains are quantified by using the refined spectrophotometric method by Stintzing et al. (*24*) or closely related protocols. The extinction coefficient for betanin is used for the betacyanins, whereas a mean extinction coefficient is used for the numerous betaxanthins. Analyses of ulluco extracts by this spectrophotometric method gave total betacyanin levels that gave a strong linear correlation ($R^2 = 0.99$) with the total betacyanin LC peak areas. Total betaxanthin levels in ulluco tubers analyzed by the spectrophotometric method gave a reasonable linear correlation with the total betaxanthin LC peak area ($R^2 = 0.93$), but the intercept was not at zero. In other words, extracts shown by LC analyses to have only low levels of betaxanthins were predicted by the spectrophotometric method to have quite high betaxanthin levels (e.g., U4n3R2, **Table 1**). This could be due to betaxanthins detected by the spectrophotometric method not being found in the LC method, but we suggest a different reason: interference from co-occurring betacyanins. The spectrophotometric method for the betaxanthins measures the absorbance at 470−480 nm, but inspection of the visible absorption spectrum for pure betacyanins, available from the diode-array detector on the LC instruments used in this study, showed that the absorbance of betacyanins at 475 nm is strong, ~35% of their absorbance at their λ_{max} of 538 nm. By contrast, the betaxanthins' absorbance at 538 nm is close to zero, so these compounds do not interfere with spectrophotometric quantification of betacyanins. Stintzing et al. (*30*) noted that LC runs were advantageous to determine the

relative ratios of yellow and red betalains to obtain the relative inherent error of the spectrophotometric method. A simple correction factor for the betacyanin absorbance at 475 nm could be calculated on the basis of the absorbance at 538 nm or on the basis of measuring visible spectra of reference compounds, or an LC method could be calibrated for full quantitative analyzes. In retrospect, the major betaxanthins in ulluco tubers were well-resolved from the betacyanins using the "betacyanin" LC method, so this one LC method could be used to quantify both classes of betalains.

The average total betacyanin levels in red-skinned ulluco tubers in this study were 53.5 ± 1.0 μg/g of fresh weight for accession U2, 70.4 ± 0.7 μg/g of fresh weight for U4n3, 63.9 ± 3.1 μg/g of fresh weight for U4n4, and 41.2 ± 5.6 μg/g of fresh weight for U15. Campos et al. (*12*) reported 64 μg/g of fresh weight total betacyanins in the one red-skinned ulluco tuber that they analyzed. The yellow ulluco accessions gave total betaxanthin levels (not affected by betacyanin interference with spectrophotometric measurements) of 42.5 ± 7.7 μg/g of fresh weight for accession U3 and 49.6 ± 5.6 μg/g of fresh weight for U9. Campos et al. (*12*) reported total betaxanthins ranging from 22 to 96 μg/g of fresh weight in the ulluco tubers that they analyzed, figures that compare well with ours. These levels of betacyanins and betaxanthins in ulluco tubers are similar to those found in Swiss chard: betacyanins 51.1 ± 0.8 μg/g of fresh weight in purple petioles; and betaxanthins 49.7 ± 0.4 μg/g of fresh weight in yellow petioles (*24*). However, betalain levels are much higher in beetroot, especially in lines selectively bred for high pigment concentrations, with betacyanin levels of 1000 μg/g of fresh weight or higher (*31*).

Ulluco has been confirmed as a member of the colorful palette of betalain-containing plants. Despite the possible health benefits of betalains, there are very few sources of these pigments in most diets. Ulluco is a potential new source, which as a starchy tuber might be consumed in larger amounts than beetroots or Swiss chard. The higher levels of betacyanins in the tetraploid accessions (**Figure 4**) suggest that selective breeding of ulluco could give increased pigment levels, as has been achieved for the betalains in beetroot (*31*). Seven different accessions were tested in the present study, but some 450 different ulluco accessions are maintained at the International Potato Centre in Lima, Peru (*2*). There may remain numerous ulluco with different betalain pigmentation patterns awaiting discovery.

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Supporting Information Available: Supporting Information contains chromatograms for the identification of betaxanthins, data on the isomerization of betacyanins and a correlation plot for the levels of betacyanins and betaxanthins determined by LC and Vis-spectrophotometry. This material is available free of charge via the Internet at http://pubs.acs.org.

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