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Antioxidant Profiling of Native Andean Potato Tubers (*Solanum tuberosum* L.) Reveals Cultivars with High Levels of β-Carotene, α-Tocopherol, Chlorogenic Acid, and Petanin

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The antioxidant profile of 23 native Andean potato cultivars has been investigated from a human nutrition perspective. The main carotenoid and tocopherol compounds were studied using high-performance liquid chromatography coupled with a diode array detector (HPLC-DAD) and a fluorescence detector, respectively, whereas polyphenols (including anthocyanins in colored tubers) were identified by means of both HPLC−mass spectrometry and HPLC-DAD. Antioxidant profiling revealed significant genotypic variations as well as cultivars of particular interest from a nutritional point of view. Concentrations of the health-promoting carotenoids, lutein and zeaxanthin, ranged from 1.12 to 17.69 μg g^{-1} of dry weight (DW) and from 0 to 17.7 μg g^{-1} of DW, with cultivars 704353 and 702472 showing the highest levels in lutein and zeaxanthin, respectively. Whereas β-carotene is rarely reported in potato tubers, remarkable levels of this dietary provitamin A carotenoid were detected in 16 native varieties, ranging from 0.42 to 2.19 μg g^{-1} of DW. The amounts of α-tocopherol found in Andean potato tubers, extending from 2.73 to 20.80 μg g^{-1} of DW, were clearly above the quantities generally reported for commercial varieties. Chlorogenic acid and its isomers dominated the polyphenolic profile of each cultivar. Dark purple-fleshed tubers from the cultivar 704429 contained exceptionally high levels of total anthocyanins (16.33 mg g^{-1} of DW). The main anthocyanin was identified as petanin (petunidin-3-*p*-coumaroyl-rutinoside-5-glucoside). The results suggest that Andean potato cultivars should be exploited in screening and breeding programs for the development of potato varieties with enhanced health and nutritional benefits.

KEYWORDS: Potato; Andean tuber; *Solanum tuberosum*; antioxidants; carotenoids; β-carotene; zeaxanthin; petanin; chlorogenic acid; polyphenols; α-tocopherol

INTRODUCTION

Dietary antioxidants include ascorbate (vitamin C), tocopherols (vitamin E), carotenoids, and polyphenols. They are believed to play a key role in the body's defense system against reactive oxygen species (ROS), which are known to be involved in the pathogenesis of aging and many degenerative diseases such as cardiovascular diseases and cancers.

Carotenoids are yellow to red lipophilic pigments synthesized by all photosynthetic organisms, including plants, and also by some non-photosynthetic bacteria and fungi. Two classes of carotenoids are found in nature: carotenes, such as β-carotene, which are linear hydrocarbons cyclized at one or both ends of the molecule, and xanthophylls, such as lutein and zeaxanthin, which are oxygenated derivatives of carotenes. In plants, carotenoids have functional roles in development, photosynthesis, and membrane stability (*1*). In humans, dietary carotenoids are associated with health benefits. The provitamin A activity of carotenoids with a β-ring end group (β-carotene, β-cryptoxanthin) is well-known and of nutritional importance (*2*). Carotenoids such as lutein and zeaxanthin have no provitamin A activity but provide protection against age-related macular degeneration (*3*). Furthermore, epidemiological studies have shown that a diet rich in carotenoids can prevent the onset of certain cancers and cardiovascular diseases (*4*).

Vitamin E includes four tocopherol and four tocotrienol compounds. In plants and animals, these molecules prevent the propagation of lipid peroxidation by scavenging lipid peroxyl

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radicals (*5*, *6*). Although α-tocopherol is the biologically most active form in the human body and is well-known for its antioxidant and antiatherosclerotic properties (*6*), the latest studies highlight the importance of the presence of both δ- and γ- in addition to α-tocopherols to achieve optimal biological effects (*7*).

Polyphenols are secondary metabolites produced in plants and are typically involved in plant defense mechanisms against ultraviolet radiation or aggression by pathogens. In humans, evidence for their role in the prevention of degenerative diseases is emerging (*8*, *9*). However, the diversity of polyphenols adds complexity to the understanding of their effects on health and their biological actions are yet poorly understood. Current research aims therefore at identifying polyphenols and unraveling their health effects. The large family of polyphenols includes phenolic acids such as benzoic and hydroxycinnamic acids, flavonoids such as flavonols and anthocyanins, stilbenes, and lignans (*10*). Along with carotenoids, anthocyanins impart color in plants. In particular, anthocyanins are hydrophilic pigments responsible for the red, purple, and blue hues in most flowers, grasses, fruits, vegetables, and grains. The association of coloring properties and potential health benefits in anthocyanins (*11*) makes them very attractive molecules for the food industry, even though their characteristics depend crucially on their chemical structure (*12*).

Humans are not capable of synthesizing these antioxidant compounds de novo; thus, their presence in human tissues relies entirely on their occurrence in the diet. Significant levels of hydrophilic antioxidants, that is, polyphenols (*13–15*) and vitamin C (*16*), and moderate levels of lipophilic carotenoids (*17–19*) and vitamin E (*20*) have been reported in the staple crop potato. Owing to its high level of consumption throughout the world, the potato could be an ideal carrier of health-promoting phytochemicals. Besides, recent reports have highlighted that the potato may significantly contribute to the antioxidant dietary intake (*16*, *20*, *21*) and is likely to provide health benefits (*22*).

Promising routes for enhancing the quality of staple crops include biotechnologies and conventional plant breeding. Although many efforts to improve potato carotenoid (*23–25*) or phenolic contents (*26*) are currently made through transgenic approaches, natural variation in the potato germplasm may also offer nutritionally interesting potato genotypes. Of particular interest are the very diverse native Andean potato landraces, which are particularly adapted to highland environments. They have been shown to have more genetic diversity as compared to modern potato varieties (*27*) and, so far, are largely unexplored from a nutritional point of view. A previous study performed by our group (*21*) highlighted the high dietary antioxidant diversity offered by native Andean genotypes in terms of vitamin C, polyphenols, and carotenoids. In that first paper, a genetically diverse collection of 74 native cultivars showed wide variabilities in total carotenoid and phenolic contents, but the identification and quantification of individual compounds were not performed. Consequently, the present work considers the characterization of these molecules as well as tocopherols in a selection of 23 genotypes with contrasting carotenoid and phenolic contents. Precisely, the main carotenoid and tocopherol compounds were studied by using high-performance liquid chromatography coupled with a diode array detector (HPLC-DAD) and a fluorescence detector (HPLC-FLD), respectively, whereas polyphenols (including anthocyanins in colored tubers) were identified by means of both HPLC−mass spectrometry (LC-MS) and HPLC-DAD.

MATERIALS AND METHODS

Chemicals. Solvents (of analytical or HPLC grade as required) were obtained from VWR International (Leuven, Belgium). Carotenoid standards (lutein, neoxanthin, violaxanthin, zeaxanthin, antheraxanthin, β-cryptoxanthin, β-carotene, and lutein-5,6-epoxide) were purchased from Carotenature (Lupsingen, Switzerland). Tocopherol standards (α-, γ-, and δ-tocopherols) and polyphenols commonly found in nature (chlorogenic acid, caffeic acid, *p*-coumaric acid, vanillic acid, ferulic acid, protocatechuic acid, gallic acid, tyrosine, tryptophan, quercetin, quercetin-3-glucoside, kaempferol, catechin, epicatechin, and rutin) were obtained from Sigma-Aldrich (St. Louis, MO). Anthocyanidins (delphinidin, pelargonidin, petunidin, peonidin, cyanidin, and malvidin) as well as kaempferol-3-rutinoside were purchased from ExtraSynthese (Genay, France). Petanin (petunidin-3-*p*-coumaroyl-rutinoside-5-glucoside) was purchased from Polyphenols Laboratories AS (Sandnes, Norway).

Plant Materials. On the basis of previous results (*21*), 23 native potato cultivars with contrasting total carotenoid and total phenolic contents were selected for the present work. Growth conditions, tuber characteristics, and sample preparation of the native Andean cultivars have been described previously (*21*). Tuber colors are shown in **Table 1**. For each cultivar, three samples (each made of three whole unpeeled tubers from one plant) were used. Each sample was extracted and analyzed in duplicate. The selection of cultivars includes five groups of cultivars from *Solanum tuberosum*: Ajanhuiri, Andigenum, Juzepczukii, Phureja, and Stenotonum.

Carotenoid Analysis. Carotenoids were extracted according to the method reported by Morris et al. (*28*) with few modifications.

Extraction. Approximately 0.5 g of powdered freeze-dried material was mixed with 5 mL of acetone. This mixture was homogenized using a vortex for 30 s and shaken on ice for 30 min to allow extraction of the lipophilic compounds. After centrifugation at 6000*g* for 15 min, the supernatant was collected. The extraction was repeated on the pellet using the same extraction solvent. Both supernatants were combined, evaporated to dryness in a SpeedVac concentrator (Heto, Thermo Electron Corp., Waltham, MA), and resuspended in 2 mL of acetone. From this 2 mL of extract, 1 mL was filtered through a 0.22 μm Acrodisc GHP syringe filter and stored at −80 °C prior to HPLC analysis.

Saponification. One milliliter of the remaining sample was dried and redissolved in 4 mL of a methanolic potassium hydroxide solution (10%, w/v). The solution was then allowed to stand overnight under nitrogen at 4 °C in the dark. Carotenoids were then purified by the addition of 4 mL of diethyl ether, followed by 4 mL of saturated aqueous NaCl, until two layers formed. The organic phase was kept separately, and the aqueous phase was re-extracted with ether. The ether extracts were then pooled and washed three times with water. The samples were then dried under a gentle stream of oxygen-free nitrogen, redissolved in 1 mL of acetone, and filtered through a 0.22 μm Acrodisc GHP syringe filter.

HPLC-DAD Analysis. Identification and quantification of carotenoids were carried out as described by Wright et al. (*29*) using a Dionex HPLC ICS-2500/BioLC (Sunnyvale, CA), with an AS50 autosampler, a GS50 gradient pump, and a PAD-100 photodiode array detector (photo-DAD). The separation was performed with a Zorbax Bonus-RP column (250 × 4.6 mm i.d.; 5 μm particle size) (Agilent Technologies, Palo Alto, CA) at 40 °C. A gradient system was used involving four mobile phases. Eluent A was a solution of methanol/0.5 M ammonium acetate (80:20, v/v; pH 7.2); eluent B, acetonitrile; eluent C, water; and eluent D, ethyl acetate. The gradient elution profile was as follows: 0 min, 100% A; 2 min, 90% B, 10% C; 2.6 min, 81% B, 9% C, 10% D; 10 min, 76% B, 10% C, 14% D; 11 min, 70% B, 20% D, 10% C; 17.6 min, 58.5% B, 6.5% C, 35% D; 20 min, 27.9% B, 3.1% C, 69% D; 22 min, 90% B, 10% C; 25 min, 100% A; 25–30 min, 100% A, re-equilibration time. The injection volume for all samples was 20 μL. Simultaneous monitoring was set at 442 nm (violaxanthin and neoxanthin), 447 nm (lutein and antheraxanthin), and 453 nm (zeaxanthin, β-cryptoxanthin, and β-carotene) for quantification at a flow rate of 1.0 mL min^{-1}. Carotenoid compounds were identified by their retention time and spectral data as compared to standards and

Table 1. Carotenoid and α-Tocopherol Contents in Tubers of 23 Native Andean Cultivars [Mean Values Represent Analyses of Three Samples from Three Different Plants ($n = 3$), Each Assayed in Duplicate]

genotype	skin and flesh color[a]	carotenoids[b] (μg g^{-1} of dry wt) neo	viola	ant	lutein	zea	β-car	esters	total	tocopherols (μg g^{-1} of dry wt) α
Ajanhuiri group										
702802-Jancko Ajawiri	W/C	1.52 ± 0.38	1.97 ± 1.30	nd[c]	7.60 ± 1.92	1.80 ± 0.35	0.70 ± 0.37	1.79	15.38	9.94 ± 2.57
704229-Jancko Anckanchi	W/W	1.85 ± 0.19	0.46 ± 0.12	nd	3.87 ± 0.18	1.14 ± 0.14	nd	nd	7.32	10.77 ± 0.40
Andigenum group										
700347-SS-2613	Y/C	1.17 ± 0.23	1.62 ± 0.46	0.48 ± 0.18	5.98 ± 0.62	1.22 ± 0.19	1.71 ± 0.20	0.07	12.25	14.06 ± 5.23
702316-Pulu	P/Cp	2.95 ± 1.06	0.98 ± 0.19	nd	9.28 ± 1.90	1.83 ± 0.36	0.92 ± 0.50	2.09	18.06	20.80 ± 8.74
702477-Yana Puma Maqui	DP/Cp	2.47 ± 0.27	1.51 ± 0.33	nd	4.95 ± 0.58	2.00 ± 0.44	0.63 ± 0.18	1.22	12.78	6.80 ± 1.26
702535-Sipancachi	Wp/Y	2.51 ± 0.58	4.91 ± 1.12	0.35 ± 0.12	7.28 ± 1.21	1.11 ± 0.36	0.42 ± 0.13	2.26	18.85	4.44 ± 1.24
702568-Pichea Papa	Py/C	1.10 ± 0.37	0.59 ± 0.16	nd	1.74 ± 0.38	1.13 ± 0.25	nd	0.79	5.35	2.73 ± 0.27
703248-Wila Huaka Lajra	Ry/C	0.83 ± 0.15	1.67 ± 0.74	0.43 ± 0.15	1.73 ± 0.89	0.68 ± 0.41	nd	1.99	7.33	3.10 ± 0.89
703739-Lisan	Y/C	2.40 ± 0.32	0.65 ± 0.12	nd	11.77 ± 1.70	1.69 ± 0.20	1.38 ± 0.29	0.39	18.28	11.95 ± 1.04
703750-Carganaca	Pr/Wp	2.10 ± 0.49	1.76 ± 0.70	0.34 ± 0.55	9.14 ± 0.87	2.06 ± 0.19	1.11 ± 0.35	nd	16.51	10.62 ± 1.03
703905-Huata Colorada	Py/Y	3.39 ± 0.48	2.73 ± 1.28	0.28 ± 0.09	14.45 ± 5.16	1.43 ± 0.39	1.99 ± 0.59	11.91	36.18	12.51 ± 3.49
704078-Malcachu	Yp/W	1.51 ± 0.35	1.59 ± 0.59	nd	9.35 ± 2.93	1.64 ± 0.12	2.00 ± 0.27	1.81	17.90	5.11 ± 1.62
704353-Puma	Py/Y	4.86 ± 1.43	6.92 ± 2.77	0.64 ± 0.17	17.69 ± 2.47	1.54 ± 0.66	2.19 ± 0.55	5.11	38.95	5.86 ± 1.64
704429-Guincho Negra	DP/P	1.41 ± 0.20	1.93 ± 0.65	0.28 ± 0.09	6.46 ± 0.66	1.62 ± 0.44	1.55 ± 0.32	3.09	16.35	9.52 ± 2.55
704437-Chata Colorada	Yr/W	1.06 ± 0.15	0.57 ± 0.24	nd	3.69 ± 0.42	1.14 ± 0.16	nd	1.96	8.41	4.99 ± 0.92
704828-Wila Immilla	Ry/Y	1.94 ± 0.41	0.69 ± 0.21	nd	12.78 ± 2.01	2.14 ± 0.31	1.49 ± 0.26	0.64	19.67	7.29 ± 0.77
704865-Holendesa	Y/C	1.07 ± 0.15	0.90 ± 0.42	0.29 ± 0.12	6.75 ± 1.71	1.70 ± 0.51	1.79 ± 0.77	0.42	12.92	5.14 ± 1.80
704916-Coyu	Y/Cp	0.71 ± 0.12	0.86 ± 0.10	nd	5.77 ± 1.13	1.23 ± 0.34	0.67 ± 0.22	1.10	10.34	4.43 ± 0.90
Juzepczukii group										
702305-Chimi Lucki	W/W	0.50 ± 0.22	0.14 ± 0.07	nd	1.12 ± 0.25	trace[d]	nd	nd	1.78	9.95 ± 1.54
703258-Laram Canchali	Pw/W	0.36 ± 0.05	0.12 ± 0.02	nd	1.41 ± 0.34	0.51 ± 0.05	nd	nd	2.40	6.89 ± 0.42
Phureja group										
701570-Chaucha	P/Y	5.31 ± 0.65	6.56 ± 1.97	0.41 ± 0.03	10.57 ± 1.35	0.76 ± 0.23	0.75 ± 0.15	7.67	32.04	15.61 ± 0.58
Stenotonum group										
702472-Amarilla del Centro	Y/Y	2.44 ± 0.39	13.29 ± 3.53	9.97 ± 1.89	7.99 ± 2.63	17.70 ± 3.73	1.48 ± 0.69	1.91	54.78	14.55 ± 3.07
702961-Garhuash Pashon	O/Y	2.71 ± 0.60	4.23 ± 1.16	0.81 ± 0.13	14.43 ± 0.69	0.60 ± 0.20	nd	4.71	27.49	12.19 ± 1.98

[a] Primary (in capital) and secondary skin color/primary (in capital) and secondary flesh color: DP, dark purple; P, purple; R, red; O, orange; Y, yellow; C, cream; W, white. [b] Abbreviations: Neo, neoxanthin; Viola, violaxanthin; Ant, antheraxanthin; Zea, zeaxanthin; β-car, β-carotene; Esters, total esterified carotenoids. [c] Not detected. [d] Present in trace amount (<0.4 μg g^{-1}).

were quantified using six-point calibration curves. Carotenoid contents were expressed in micrograms per gram of dry weight (DW). The quantity of esters was estimated as the difference in carotenoids between the native and saponified extract. The total carotenoid contents were calculated by summing concentrations of all compounds.

Tocopherol Analysis. The extraction of tocopherols was similar to the method described for carotenoids. The analysis of α-, γ-, and δ-tocopherols by HPLC was adapted from that of Hoeler et al. (*30*) and was performed using a Dionex Summit system (Sunnyvale, CA) equipped with a P580 gradient pump, a GINA 50 autosampler, a Jasco FP-920 fluorescence detector (Tokyo, Japan), a UVD 340S DAD, and an external Bio-Rad column heater (Hercules, CA). A 20 μL aliquot was injected onto a Zorbax Bonus-RP column (150 × 4.6 mm i.d.; 5 μm particle size) (Agilent Technologies, Palo Alto, CA). The mobile phase was methanol/water (95:5, v/v). Tocopherols were eluted isocratically at a flow rate of 1.2 mL min^{-1} and at 30 °C. Quantification was accomplished at excitation and emission wavelengths of 290 and 325 nm, respectively, by comparing integrated chromatographic peak areas from the samples to peak areas of known amounts of tocopherols. Tocopherol content was expressed in micrograms per gram of DW.

Polyphenol Analysis. *Extraction.* Approximately 150 mg of powdered freeze-dried material was mixed with 1.5 mL of methanol/water/acetic acid (80:19.5:0.5, v/v/v). This mixture was homogenized using a vortex for 30 s and shaken for 30 min at 4 °C. After centrifugation at 9000*g* for 10 min at 4 °C, the supernatant was collected. Two additional extractions were done on the residue using the same extraction solvent. The supernatants were pooled and evaporated to dryness in a SpeedVac concentrator (Heto, Thermo Electron Corp., Waltham, MA). Polyphenols were resuspended in 500 μL of water and filtered through a 0.45 μm Acrodisc PVDF syringe filter prior to injection.

HPLC-DAD Analysis. The quantification of the phenolic compounds was carried out using a Dionex Summit system (Sunnyvale, CA) equipped with a P580 gradient pump, a GINA 50 autosampler, a UVD 340S DAD, and an external Bio-Rad column heater (Hercules, CA). A 20 μL aliquot was injected onto a Nucleodur C18 Pyramid column (250 × 4 mm i.d.; 5 μm particle size) (Macherey-Nagel, Düren, Germany). The mobile phases were (A) water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid. The flow rate was 1.0 mL min^{-1}, and the column temperature was 40 °C. The 95 min gradient was as follows: 0–10 min, 0–9% B; 10–40 min, 9–13% B; 40–80 min, 13–35% B; 80–82 min, 35–100% B; 82–87 min, 100% B linear; 87–90 min, 100–0% B; 90–95 min, 0% B, re-equilibration time. Simultaneous monitoring was set at 254 nm (protocatechuic and vanillic acids, rutin, kaempferol-3-rutinoside), 280 nm (tyrosine and tryptophan), and 320 nm (caffeic, ferulic, neochlorogenic, chlorogenic, and cryptochlorogenic acids) for quantification. Other caffeoylquinic acids (CQA) such as neochlorogenic (3-CQA) and cryptochlorogenic (4-CQA) acids were quantified as chlorogenic acid (5-CQA) equivalents. Polyphenols were expressed in micrograms per gram of DW.

HPLC-ESI/MS Analysis. Identification of the main phenolic compounds was performed by LC-MS using a Finnigan single-quadrupole MSQ MS detector (ThermoFinnigan, San Jose, CA) equipped with an electron spray ionization (ESI) probe. The instrument was operated at the following settings: nitrogen gas flow, 12 L min^{-1}; capillary voltage, 3.5 kV; cone voltage, 20 and 60 V; capillary temperature, 500 °C. Column and elution conditions were as described above for the HPLC-DAD analysis. Spectra were recorded in positive ion mode between 100 and 1000 atomic mass units (amu).

Total Anthocyanin Quantification. Total anthocyanins were measured using the pH differential method (*31*). Absorbance was measured at 520 and 700 nm in pH 1.0 and 4.5 buffers on the crude phenolic extract using a Beckman DU 800 spectrophotometer (Fullerton, CA). Petanin (petunidin-3-*p*-coumaroyl-rutinoside-5-glucoside) was used for the standard curve, and total anthocyanins were expressed as petanin equivalents.

Anthocyanin Purification. The purification of anthocyanins was carried out using solid-phase extraction cartridges as described by

Rodriguez-Saona and Wrolstad (*32*) and Chirinos et al. (*33*). Briefly, the polyphenolic aqueous extract was passed through a C18 cartridge (Varian, Palo Alto, CA) previously conditioned with methanol and 0.01% aqueous HCl. Sugars, acids, phenolic acids, and other interfering substances were eluted by rinsing with 0.01% aqueous HCl followed by ethyl acetate. Anthocyanins, retained on the cartridge, were then eluted with acidified methanol (0.01% HCl). The eluate was concentrated under vacuum, redissolved in methanol, and kept at −80 °C prior to HPLC analysis.

HPLC-DAD Analysis of Anthocyanins. HPLC-DAD analysis was used to determine the spectroscopic characteristics of the native anthocyanins as well as to identify all of the anthocyanidins present in the hydrolyzed anthocyanin extract. This analysis was performed using the system and column already described for polyphenol quantification. The mobile phases were (A) water and (B) acetonitrile, both with 0.1% trifluoroacetic acid, with a flow rate of 1.0 mL min^{-1} at 40 °C. The 50 min gradient was as follows: 0–10 min, 10% B; 10–30 min, 10–40% B; 30–35 min, 40–100% B; 35–40 min, 100% B; 45–50 min, 100–10% B. Anthocyanin glucosides and anthocyanidins present in the samples were determined after alkaline and acid hydrolyses, respectively, according to the procedure proposed by Durst and Wrolstad (*34*). Sugars released during acid hydrolysis were also evaluated by high-performance anion exchange chromatography coupled to pulsed amperometric detection (HPAEC-PAD) as previously described in Guignard et al. (*35*).

HPLC-ESI/MS Analysis of Anthocyanins. Identification of the main anthocyanins present in four high anthocyanin-ranking cultivars was accomplished using the system and column already described for polyphenol identification. The instrument settings were as follows: cone voltage, 80 and 150 V; capillary temperature, 450 °C. As the mobile phase used for HPLC-DAD was not adequate for the mass spectrometer, a new eluent was developed for this analysis. The solvents were (A) water with 5% formic acid and (B) acetonitrile. Column temperature was 40 °C, and injection volume was 10 μL. The gradient elution was as follows: 0–7.5 min, 7% B, flow rate = 1 mL min^{-1}; 7.5–8 min, 7–17% B, flow rate = 1–0.5 mL min^{-1}; 8–30 min, 17% B; 30–35 min, 17–30% B; 35–39.5 min, 30% B, flow rate = 0.5–1 mL min^{-1}; 39.5–40 min, 30–100% B; 40–45 min, 100% B; 45–47 min, 100–7% B; 47–50 min, 7% B, re-equilibration time.

Statistical Analyses. Pearson correlation coefficients were determined on log-transformed data to evaluate relationships between variables. Principal component analysis (PCA) was performed on centered and standardized data to compare the antioxidant profile of the different potato genotypes. For that purpose, the computer program Canoco 4.0 for Windows (Center for Biometry, Wageningen, The Netherlands) and SigmaPlot 7.101 software (Systat Software Inc., San Jose, CA) were used.

RESULTS AND DISCUSSION

Carotenoid Compounds. The carotenoid pattern of the potato extracts was dominated by lutein, neoxanthin, violaxanthin, zeaxanthin, and antheraxanthin, followed by β-cryptoxanthin and β-carotene as minor components. Their identification was confirmed by comparison of their retention times and UV spectra with those of authentic standards. The quantitative results of all 23 cultivars under investigation are presented in **Table 1**. β-Cryptoxanthin was detected in only one potato genotype (702472-Amarilla del Centro) and in trace amount (<0.1 μg g^{-1} of DW); thus, it was not included in the table. Lu et al. (*17*) found lutein-5,6-epoxide as the main carotenoid compound in the tubers of their hybrid population of *Solanum phureja-stenotonum*. This standard was also included in our study but was not detected in any cultivar, as previously reported in other studies (*19, 28*). The total carotenoid contents ranged from 1.78 μg g^{-1} of DW in 702305-Chimi Lucki (Juzepczukii group) to 54.78 μg g^{-1} of DW in 702472-Amarilla del Centro (Stenotonum group). A strong correlation was found ($r = 0.96$, $p < 0.01$) between the total carotenoid content assessed spectrophotometrically at 450 nm in our previous study (*21*) and the amount determined here by summing all of the individual carotenoid compounds quantified by HPLC-DAD. However, the first method slightly underestimated the total carotenoid content in the highest ranking cultivars (on average 4 μg g^{-1} of DW less).

The predominant carotenoid compound in our potato tubers was lutein. The contents varied greatly among the 23 potato genotypes and ranged from 1.12 to 17.69 μg g^{-1} of DW, with a mean value of 7.64 μg g^{-1} of DW. 701570-Chaucha (Phureja group), 702961-Garhuash Pashon (Stenotonum group), and 704353-Puma or 703905-Huata Colorada (Andigenum group) were all high-lutein-containing tubers (>10 μg of lutein g^{-1} of DW). Such levels have already been described in hybrid populations of *Solanum phureja-stenotonum* (*17*) or in transgenic tubers (*24*), yet, to our knowledge, the high lutein content observed in cultivars of the Andigenum group has never been reported in tetraploid potatoes. Whereas lutein dominated the carotenoid profile in most potato cultivars and accounted for an average of 47% of the total carotenoid content, the highest total carotenoid-ranking cultivar, namely, 702472-Amarilla del Centro, was differently balanced. Indeed, in this genotype, lutein accounted for only 14% of the total carotenoid content. In addition, the levels in zeaxanthin and antheraxanthin in this genotype were exceptionally high, representing >32 and >18% of the total carotenoid content, respectively. Similar zeaxanthin and antheraxanthin concentrations have also been reported in high-carotenoid-accumulating genotypes from the Phureja group (*28*) or in hybrid populations of Phureja−Stenotonum (*19*). In addition, a transgenic strategy has been proposed to increase the zeaxanthin level in potato (*23*), reaching values in peeled mini tubers 2-fold higher than in 702472-Amarilla del Centro.

Interestingly, remarkable levels of β-carotene were detected in 16 potato cultivars from our collection, with concentrations ranging from 0.42 to 2.19 μg g^{-1} of DW. The presence of β-carotene was confirmed by spiking the sample extracts with authentic β-carotene standard (data not shown). β-Carotene-containing tubers have been reported in the literature, but either in poor amount in potato germplasm [approximately 0.15 μg g^{-1} of DW, recalculated from Nesterenko et al. (*19*)] or at higher concentrations as a result of transgenic modifications [10.3 μg g^{-1} of DW (*24*)]. It is therefore the first time that such high β-carotene levels (>2 μg g^{-1} of DW) are described in the potato germplasm. Every cultivar from our collection with >10 μg of total carotenoids g^{-1} of DW did contain β-carotene, with the surprising exception of one high-carotenoid-ranking cultivar from the Stenotonum group, 702961-Garhuash Pashon, which did not present any.

Alkaline saponification revealed the presence of carotenoid esters in various amounts in high-carotenoid-ranking cultivars, whereas no esters were detected in low-ranking ones. High levels were reached in 703905-Huata Colorada (11.91 μg g^{-1} of DW) from the Andigenum group and 701570-Chaucha (7.67 μg g^{-1} of DW) from the Phureja group, contributing ~33 and ~24% of the total carotenoid content, respectively. Therefore, as already stated by Breithaupt and Bamedi (*18*), carotenoid esters have to be regarded as quantitatively significant in certain potato genotypes. The range of ester concentrations observed in our study is similar to the results reported in the literature (*18, 28*). Carotenoid esters were mainly present as violaxanthin, lutein, and zeaxanthin esters. Myristic and palmitic acids have been reported to be the main fatty acids bound to potato carotenoids (*18*).

More than 600 carotenoids and related compounds are identified in human food products from animal or plant origin.

Table 2. Polyphenol Contents in Tubers of 23 Native Andean Cultivars [Mean Values Represent Analyses of Three Samples from Three Different Plants ($n = 3$), Each Assayed in Duplicate]

	aromatic amino acids (μg g^{-1} of DW)		phenolic acids (μg g^{-1} of DW)				flavonoids (μg g^{-1} of DW)		
genotype	tyrosine	tryptophan	3-CQA[a]	4-CQA[b]	5-CQA[c]	caffeic acid	rutin	kaempf-3-rut[d]	total anthocyanins
Ajanhuiri group									
702802-Jancko Ajawiri	1565 ± 374	503 ± 205	28 ± 15	101 ± 77	174 ± 63	22 ± 23	115 ± 19	2 ± 2	nd[e]
704229-Jancko Anckanchi	1519 ± 257	1145 ± 79	19 ± 2	50 ± 6	325 ± 28	17 ± 2	29 ± 2	3 ± 1	nd
Andigenum group									
700347-SS-2613	4003 ± 532	830 ± 389	55 ± 49	96 ± 72	224 ± 117	22 ± 5	157 ± 27	66 ± 22	nd
702316-Pulu	2191 ± 1214	369 ± 307	204 ± 131	470 ± 237	1586 ± 592	33 ± 13	nd	nd	2931 ± 1648
702477-Yana Puma Maqui	5247 ± 1273	1122 ± 579	48 ± 7	152 ± 18	2701 ± 910	106 ± 32	nd	nd	2262 ± 1116
702535-Sipancachi	1246 ± 285	205 ± 56	9 ± 3	23 ± 9	264 ± 53	9 ± 3	13 ± 10	1 ± 1	nd
702568-Pichea Papa	928 ± 477	234 ± 142	43 ± 8	50 ± 14	226 ± 83	22 ± 7	9 ± 2	nd	nd
703248-Wila Huaka Lajra	884 ± 580	237 ± 97	14 ± 9	28 ± 16	216 ± 101	46 ± 24	23 ± 5	3 ± 0	14 ± 1
703739-Lisan	1803 ± 461	305 ± 128	47 ± 18	161 ± 41	459 ± 90	31 ± 10	191 ± 34	224 ± 25	nd
703750-Carganaca	1977 ± 212	320 ± 47	28 ± 4	131 ± 24	2732 ± 805	63 ± 22	166 ± 64	78 ± 51	1919 ± 778
703905-Huata Colorada	473 ± 120	143 ± 14	63 ± 21	138 ± 83	307 ± 255	62 ± 12	25 ± 11	54 ± 18	52 ± 27
704078-Malcachu	1022 ± 86	347 ± 132	28 ± 11	54 ± 19	499 ± 193	29 ± 9	43 ± 41	23 ± 3	nd
704353-Puma	446 ± 227	361 ± 319	96 ± 31	464 ± 199	1455 ± 678	48 ± 29	14 ± 1	10 ± 4	50 ± 9
704429-Guincho Negra	2356 ± 756	180 ± 62	150 ± 85	768 ± 341	12746 ± 5898	143 ± 46	nd	nd	16330 ± 4846
704437-Chata Colorada	1444 ± 340	326 ± 66	15 ± 3	43 ± 10	175 ± 130	37 ± 24	nd	2 ± 0	nd
704828-Wila Immilla	3414 ± 230	387 ± 39	48 ± 26	123 ± 83	320 ± 213	24 ± 10	nd	77 ± 43	nd
704865-Holendesa	1615 ± 621	273 ± 128	61 ± 64	121 ± 100	252 ± 198	12 ± 5	22 ± 10	16 ± 4	nd
704916-Coyu	2380 ± 842	452 ± 94	31 ± 6	91 ± 9	437 ± 101	13 ± 4	77 ± 6	227 ± 43	nd
Juzepczukii group									
702305-Chimi Lucki	1259 ± 664	285 ± 178	6 ± 4	14 ± 10	292 ± 152	35 ± 16	nd	nd	nd
703258-Laram Canchali	525 ± 123	371 ± 79	7 ± 2	21 ± 7	282 ± 103	49 ± 21	8 ± 2	nd	24 ± 17
Phureja group									
701570-Chaucha	5074 ± 490	497 ± 51	47 ± 31	136 ± 47	593 ± 63	15 ± 3	126 ± 59	7 ± 5	49 ± 16
Stenotonum group									
702472-Amarilla del Centro	1191 ± 657	221 ± 65	22 ± 11	103 ± 48	560 ± 134	24 ± 9	38 ± 16	5 ± 2	nd
702961-Garhuash Pashon	2594 ± 844	439 ± 132	10 ± 3	30 ± 4	364 ± 34	27 ± 9	7 ± 2	7 ± 4	nd

[a] 3-*O*-Caffeoylquinic acid or neochlorogenic acid. [b] 4-*O*-Caffeoylquinic acid or cryptochlorogenic acid. [c] 5-*O*-Caffeoylquinic acid or chlorogenic acid. [d] Kaempferol-3-*O*-rutinoside. [e] Not detected.

However, only a few are detected in human plasma, the most abundant compounds being β-carotene, lutein, lycopene, α-carotene, β-cryptoxanthin, and zeaxanthin (*36*). The preferential bioavailability of these compounds might be related to their favorable chemical structure. Besides, epoxide-containing carotenoids such as violaxanthin seem to be poorly absorbed. It has been suggested that these compounds are either chemically unstable under acidic conditions in the stomach or enzymatically modified in the intestinal cells, preventing their presence in the blood circulation (*37*). No data are available concerning the absorption of antheraxanthin and neoxanthin, but their chemical structure containing an epoxide suggests a weak absorption as assumed for violaxanthin. Bioavailability of carotenoid esters has been shown to be equivalent to free carotenoids because an effective ester hydrolysis occurs in the human gastrointestinal tract (*36*).

Lutein, zeaxanthin, and β-carotene are well-known for their health-promoting properties. Lutein and zeaxanthin may have a protective effect against age-related macular degeneration, a major cause of blindness in the elderly (*3*). Although lutein is abundant in our diet, especially in green leafy vegetables, zeaxanthin occurs to a much lesser extent. In this respect, sources rich in zeaxanthin are of particular interest. Potato is the third main contributor (13–20%) to the zeaxanthin dietary intake after citrus fruits and green vegetables (*38*). Therefore, including tubers of 702472-Amarilla del Centro in the diet (with a zeaxanthin content of 17.7 μg g^{-1} of DW), instead of commercial varieties (0.8 μg g^{-1} of DW (*38*)) or even of its native Andean counterparts (on average 1.3 μg g^{-1} of DW), could significantly increase the zeaxanthin dietary intake in the population.

Vitamin A deficiency remains a major public health concern, emphasizing the need for a staple crop enhanced in carotenoid exhibiting vitamin A activity such as β-carotene. The FAO's Reference Nutrient Intake (RNI) for vitamin A in adults is estimated as 500 and 600 μg of retinol equivalents day^{-1}, for women and men, respectively (*2*). The bioavailability of β-carotene, that is, its vitamin A activity, depends on numerous factors (*36*). The FAO recommends a conversion factor of β-carotene to vitamin A of 6 (6 μg of β-carotene = 1 μg of retinol) (*2*), whereas other studies suggest a lower bioavailability of β-carotene and a conversion factor of 12 (*39*). Assuming that 6 (or 12) μg of β-carotene is equivalent to 1 μg of retinol, 150 g of fresh weight (FW) of 704353-Puma tubers could contain ~14 (or ~7) μg of retinol equivalents. Therefore, the consumption of these Andean tubers could make only a weak contribution to the recommended daily intake. However, these β-carotene-containing Andean tubers could be excellent candidates for breeding programs, which have shown to be very effective in increasing the carotenoid contents in potato tubers (*40*).

Tocopherol Compounds. With regard to the vitamin E analysis, α-tocopherol was the only compound identified in the potato germplasm. δ- or γ-tocopherol was not detected in any cultivar. The levels of α-tocopherol observed in the Andean potato tubers, ranging from 2.73 to 20.80 μg g^{-1} of DW (**Table 1**), were clearly above the quantities generally reported in the literature for commercial varieties [0.6−3 μg g^{-1} of DW, recalculated from Chun et al. (*41*) and Spychalla and Desborough (*42*)]. To our knowledge, it is the first time that such high α-tocopherol levels have been reported in potato tubers. To validate our analytical method, two commercial varieties were also studied for their vitamin E content. The levels observed

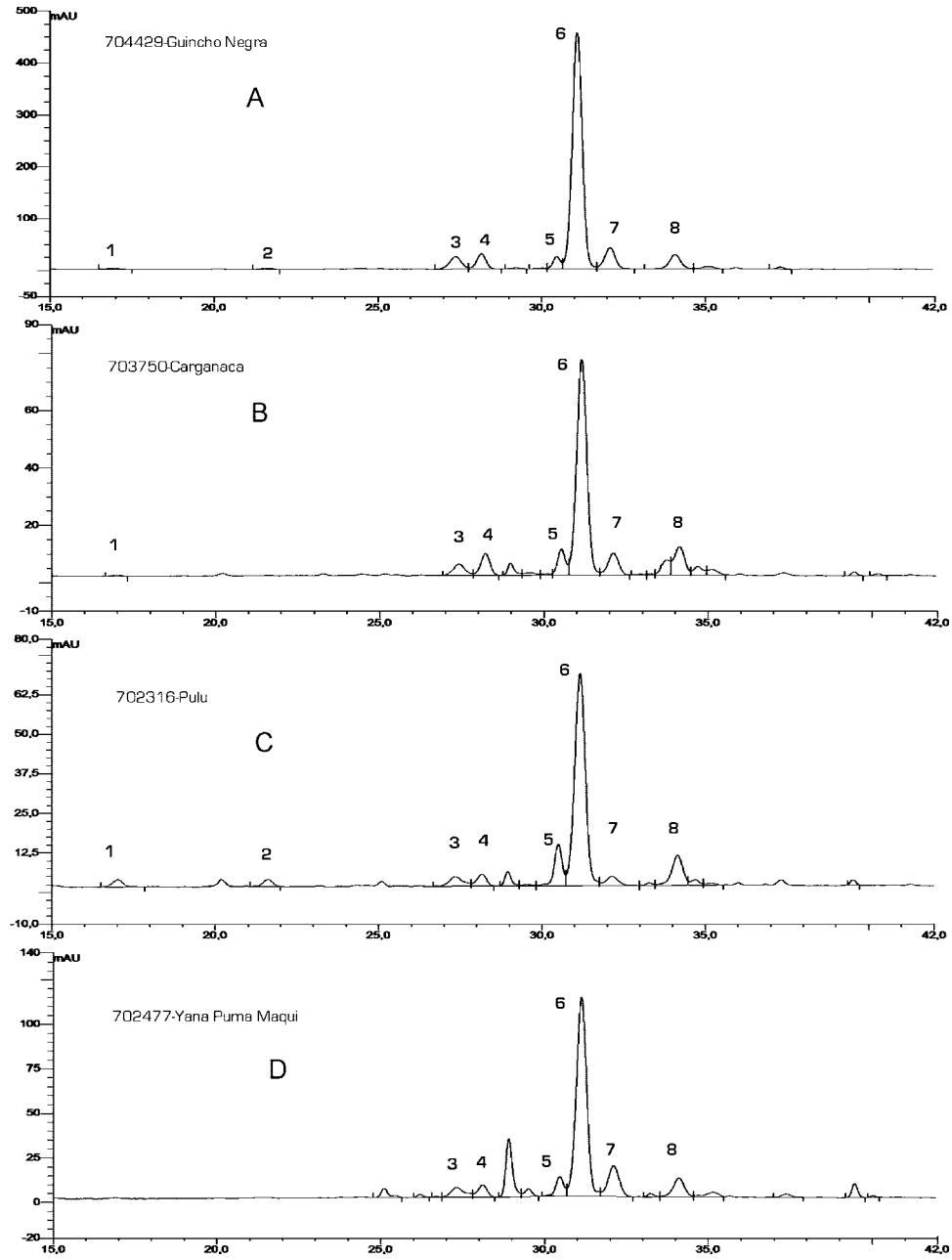


Figure 1. Anthocyanin HPLC-DAD profiles of four native Andean potato cultivars recorded at 520 nm. See **Table 3** for peak identification.

for Nicola, with a brownish skin and a pale yellow flesh, and Vitelotte, with a purple skin and a partly purple flesh, were, respectively, 0.8 and 2.3 μg of α-tocopherol g^{-1} of DW, which are in general agreement with the levels reported by other authors (*41*, *42*).

In most situations, vitamin E intake in humans is adequate and there is very little clinical evidence of deficiency. Therefore, there are no specific recommendations of the FAO concerning the vitamin E requirements. An "acceptable" daily intake of 7.5–10 mg for women and men was rather proposed (*2*). However, it is generally assumed that increases of α-tocopherol in the diet contribute to a decreased risk of chronic diseases (*6*). Improving vitamin E content in potatoes is therefore of undeniable interest. In this respect, the consumption of 150 g of FW of a high vitamin E containing potato tuber, such as 702316-Pulu, instead of traditional commercial varieties could increase the dietary vitamin E intake by 8–10%.

A positive and significant correlation was found between total carotenoids and α-tocopherol ($r = 0.5$, $p < 0.05$), indicating a positive relationship between both biosynthesis pathways. Interestingly, an increase of α-tocopherol was also noted in transgenic potato tubers containing enhanced levels of carotenoids (*23*, *24*).

Polyphenolic Compounds. HPLC-DAD profiling of the hydrophilic potato extracts revealed approximately 10 major peaks as well as several minor components. The high UV-absorbing peaks were then identified by LC-MS from their mass spectra and previous literature. These assignments could be afterward confirmed by HPLC-DAD by comparison of their UV spectra and retention times with those of authentic standards when available.

Generally, the phenolic pattern of our potato extracts was dominated by tyrosine, tryptophan, phenylalanine, chlorogenic acid, neochlorogenic acid, cryptochlorogenic acid, caffeic acid, rutin, kaempferol-3-rutinoside, and anthocyanins (in purplish tubers), followed by protocatechuic, vanillic, and ferulic acids as more minor components. The contents of the main tuber polyphenolic compounds are shown in **Table 2**.

Table 3. Spectral Characteristics of Anthocyanins Detected in Tubers of Four Potato Cultivars

peak	relative peak area (%)	molecular ion M^+ (*m*/*z*)	fragment ions (*m*/*z*)	absorbance maxima (nm)	proposed anthocyanin[a]
			704429-Guincho Negra		
1	0.32	611	465, 303	280, 521	del-3-rut
2	0.36	787	625, 479, 317	280, 533	pet-3-rut-5-glc
3	4.64	949	787, 479, 317	280, 327, 533	pet-3-caf-rut-5-glc
4	4.45	919	757, 465, 303	280, 308, 529	del-3-coum-rut-5-glc
5	3.00	903	741, 449, 287	280, 308, 536	cyan-3-coum-rut-5-glc
6	74.92	933	771, 479, 317	280, 305, 532	pet-3-coum-rut-5-glc[b]
7	7.18	963	801, 479, 317	278, 334, 531	pet-3-fer-rut-5-glc
8	5.14	917	755, 463, 301	293, 311, 523	peo-3-coum-rut-5-glc
			703750-Carganaca		
1	0.49	611	465, 303	280, 519	del-3-rut
3	4.11	949	787, 479, 317	280, 327, 528	pet-3-caf-rut-5-glc
4	6.12	919	757, 465, 303	281, 308, 527	del-3-coum-rut-5-glc
5	6.44	903	741, 449, 287	280, 305, 537	cyan-3-coum-rut-5-glc
6	66.79	933	771, 479, 317	280, 300, 530	pet-3-coum-rut-5-glc[b]
7	7.09	963	801, 479, 317	279, 330, 529	pet-3-fer-rut-5-glc
8	8.96	917	755, 463, 301	293, 314, 523	peo-3-coum-rut-5-glc
			702316-Pulu		
1	2.09	611	465, 303	280, 519	del-3-rut
2	1.80	787	625, 479, 317	280, 530	pet-3-rut-5-glc
3	3.82	949	787, 479, 317	278, 328, 531	pet-3-caf-rut-5-glc
4	3.50	919	757, 465, 303	291, 311, 530	del-3-coum-rut-5-glc
5	9.49	903	741, 449, 287	284, 308, 535	cyan-3-coum-rut-5-glc
6	67.21	933	771, 479, 317	279, 300, 531	pet-3-coum-rut-5-glc[b]
7	2.55	963	801, 479, 317	279, 331, 532	pet-3-fer-rut-5-glc
8	9.54	917	755, 463, 301	281, 309, 523	peo-3-coum-rut-5-glc
			702477-Yana Puma Maqui		
3	4.18	949	787, 479, 317	278, 333, 537	pet-3-caf-rut-5-glc
4	3.74	919	757, 465, 303	291, 311, 525	del-3-coum-rut-5-glc
5	5.28	903	741, 449, 287	280, 310, 536	cyan-3-coum-rut-5-glc
6	68.55	933	771, 479, 317	280, 300, 532	pet-3-coum-rut-5-glc[b]
7	11.37	963	801, 479, 317	279, 332, 532	pet-3-fer-rut-5-glc
8	6.87	917	755, 463, 301	280, 310, 521	peo-3-coum-rut-5-glc

[a] Abbreviations used: del , delphinidin; pet, petanin; cyan, cyanidin; peo, peonidin; glc, glucoside; rh, rhamnoside; rut, rutinoside; caf, caffeoyl; coum, *p*-coumaroyl; fer, feruloyl. [b] Identity confirmed with authentic standard.

The three aromatic amino acids (phenylalanine, tyrosine, and tryptophan) were present to a large extent in our tubers, with tyrosine being the most abundant. Unlike phenylalanine, tyrosine and tryptophan have been shown to exhibit antioxidant activity (*43*), suggesting that these compounds may contribute to the antioxidant capacity of potato phenolic extracts previously investigated (*21*). The contents of these two aromatic amino acids were therefore taken into account in this study and are also reported in **Table 2**.

Chlorogenic acid (5-CQA, 5-*O*-caffeoylquinic acid) was the predominant phenolic acid present in our potato cultivars, as has previously been reported in numerous studies on potato polyphenols (*13, 14*). Two other isomers of chlorogenic acid, neochlorogenic (3-CQA, 3-*O*-caffeoylquinic acid) and cryptochlorogenic (4-CQA, 4-*O*-caffeoylquinic acid) acids, were identified on the basis of previously established UV and mass spectra (*14, 44*). The three isomers, neo-, crypto-, and chlorogenic acids, were found in various proportions among potato genotypes, ranging between 1 and 15%, 5 and 26%, and 57 and 94% of the total chlorogenic acids, respectively. Although caffeic acid is often esterified with quinic acid to form chlorogenic acid, the aglycone also occurred in our potato tubers (9–143 μg g^{-1} of DW). Chlorogenic acid and caffeic acid were positively and significantly correlated ($r = 0.8$, $p < 0.01$), the former being on average 35-fold more present than the latter. Protocatechuic, vanillic, and ferulic acids were also identified, but their values are not reported in **Table 2** due to their low occurrence in the potato germplasm (0–11, 0–5, and 0–3 μg g^{-1} of DW, respectively).

Interestingly, it has been shown that the different chlorogenic acid isomers exhibit similar antioxidant activities (*45*) in vitro. In addition to their antioxidant properties, polyphenols may have other specific biological activities modulating the gene expression and the activity of a wide range of enzymes and cell receptors (*8, 9*). However, their properties in vivo depend on their absorption in the gut and their metabolism. It has been demonstrated that esterification of caffeic acid, as in chlorogenic acid, markedly reduced its bioavailability (*46*), but the exact mechanisms of its absorption remain unclear and need further investigations.

Concerning the chemical class of flavonoids, flavonols and anthocyanins were found as the main compounds. In particular, two identified compounds dominated the flavonol profile: rutin (quercetin-3-*O*-rutinoside) and kaempferol-3-*O*-rutinoside, with concentrations of 0–191 and 0–227 μg g^{-1} of DW, respectively. Total anthocyanins were measured spectrophotometrically on the crude phenolic extract. As expected, no anthocyanins were found in the white-skinned and -fleshed tubers. In contrast, anthocyanin contents ranging from 14 to 16330 μg g^{-1} of DW were found in their colored counterparts. Among them, four cultivars from the Andigenum group, either totally purple-fleshed (704429-Guincho Negra) or only partially purple-fleshed (702316-Pulu, 702477-Yana Puma Maqui, and 703750-Carganaca), contained very high levels of anthocyanins (1919–16330 μg g^{-1} of DW). The anthocyanin composition was therefore investigated in these four cultivars and is described below.

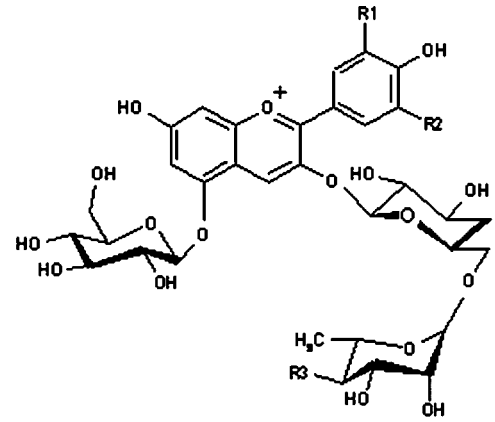
Similarly to carotenoid compounds, a strong relationship was found ($r = 0.95$, $p < 0.01$) between the total phenolic content

assessed by the Folin−Ciocalteu method described in our previous study (*21*) and the amount determined here by summing all of the individual phenolic compounds (including aromatic amino acids) quantified by HPLC-DAD. Again, as reported for carotenoids, the spectrophotometric method underestimated the total phenolic content in the highest ranking cultivars. In particular, the four purple-fleshed cultivars actually contain on average 1.7 times more phenolics than the amount predicted with the Folin−Ciocalteu method.

Anthocyanin Analysis. As mentioned above, the anthocyanin composition was determined in the tubers of four purple-fleshed potato cultivars. This was done by means of both HPLC-DAD and HPLC-ESI/MS. As can be seen in **Figure 1** and **Table 3**, the four potato genotypes contain roughly the same anthocyanins. In particular, one anthocyanin (peak 6) was predominant in each of the four profiles, representing 67–75% of the total anthocyanins. It was followed by seven minor anthocyanin compounds in various proportions as revealed by their different relative peak areas.

Relevant information about structural properties of the eight anthocyanins was first obtained from their UV–vis spectra as proposed by Giusti and Wrolstad (*31*). Acid hydrolysis revealed the presence of the aglycone petunidin as the major component in each cultivar. Delphinidin, cyanidin, and peonidin occurred also but to a much lesser extent, whereas traces of malvidin were detected in 704429-Guincho Negra, 702316-Pulu, and 702477-Yana Puma Maqui, as were traces of pelargonidin in 702316-Pulu and 703750-Carganaca (data not shown). HPAEC-PAD sugar analysis revealed the presence of glucose and rhamnose as well as of small amounts of galactose (~5% of the total hexoses in each of the four cultivars). Due to its low occurrence, the presence of galactose was not taken into account in this study. Tentatively identified on the basis of the UV–vis spectra of the anthocyanins, the presence of *p*-coumaric, caffeic, and ferulic acids as acylating compounds was confirmed after alkaline hydrolysis. The method described previously for the quantification of polyphenols was used.

MS characteristics of the native anthocyanins were combined to the gathered spectroscopic information and compared with the available data from the literature (*13*, *15*, *33*, *47–49*). The anthocyanin composition in the four cultivars could therefore be inferred as presented in **Table 3** and **Figure 2**. With the exception of peaks 1 and 2, all of the anthocyanin compounds (*3–8*) followed the same substitution pattern: -3-acylrutinoside-5-glucoside. Peak 6 was the main anthocyanin in the four samples and exhibited a molecular ion at *m*/*z* 933, which was fragmented into three major fragments at *m*/*z* 771, *m*/*z* 479, and *m*/*z* 317. The *m*/*z* 317 ion indicates that the anthocyanin was a petunidin derivative. The fragment ion *m*/*z* 771 was generated by the loss of one glucose (162 u), probably at the C5 position. The ion *m*/*z* 479 was produced by the loss of 454 u, likely resulting from the loss of one *p*-coumaroyl-rutinose unit at the C3 position [146 u + (162 u + 146 u)]. Indeed, the maximum at 305 nm in the UV–vis spectrum is indicative of the acylation of the moiety with *p*-coumaric acid. As a result, the anthocyanin was identified as petunidin-3-*p*-coumaroyl-rutinoside-5-glucoside (commonly known as petanin). The identification of this compound was further confirmed by comparison of its retention time and UV spectrum with those of a pure standard. The presence of petanin was already reported in purple-fleshed tubers from some tetraploid *Solanum tuberosum* (*13*, *15*, *47*) and diploid *Solanum stenotonum* cultivars (*48*). However, to our knowledge, this is the first time that petanin is described in tubers from the tetraploid Andigenum group.



Anthocyanin	R1	R2	R3
2	OMe	OH	OH
3	OMe	OH	caffeic acid
4	OH	OH	p-coumaric acid
5	OH	H	p-coumaric acid
6	OMe	OH	p-coumaric acid
7	OMe	OH	ferulic acid
8	OMe	H	p-coumaric acid

Figure 2. Chemical structures of the main identified anthocyanins in the four native Andean potato cultivars. Numbers refer to peak numbers reported in **Table 3**.

Peaks 3 and 7 both produced a fragment at *m*/*z* 317, indicating that they were also petunidin derivatives. They exhibited molecular ions at *m*/*z* 949 and 963, respectively. These molecular ions were fragmented into three major fragments at *m*/*z* 787, 479, and 317 and *m*/*z* 801, 479, and 317, respectively. Therefore, peaks 3 and 7 were identified as petunidin-3-caffeoyl-rutinoside-5-glucoside and petunidin-3-feruloyl-rutinoside-5-glucoside, respectively. Peaks 4, 5, and 8 revealed molecular ions at *m*/*z* 919, 903, and 917, respectively. The presence of the aglycones delphinidin (*m*/*z* 303), cyanidin (*m*/*z* 287), and peonidin (*m*/*z* 301) could, respectively, have been detected in peaks 4, 5, and 8. Besides, these three peaks followed the same substitution pattern, that is -3-*p*-coumaroyl-rutinoside-5-glucoside. In contrast to compounds **3**–**8**, peaks 1 and 2 presented a low absorbance in the UV $\lambda_{300-330nm}$ region, suggesting the absence of acylation of both anthocyanins. Whereas peak 2 presented a low absorbance at λ_{440nm}, peak 1 formed a shoulder in this spectrum region, which is characteristic of 3-glycosylation and the lack of a C5 substitution pattern. Peaks 1 and 2 showed molecular ions at *m*/*z* 611 and 787 and major fragments at *m*/*z* 465 and 303 and 635, 479, and 317, respectively. They were thus identified as delphinidin-3-rutinoside and petunidin-3-rutinoside-5-glucoside, respectively. Interestingly, in contrast to the acylated anthocyanins **3**–**8**, these two nonacylated anthocyanins were not detected in all four cultivars, stressing their low occurrence and the predominance of their acylated counterparts in Andigenum tubers. In addition, although traces of the aglycones malvidin and pelargonidin were detected after hydrolysis, their derivatives could not be identified in MS.

Anthocyanins present in our Andigenum potato cultivars represent promising natural colorants for the food industry. Indeed, they are almost all acylated with hydroxycinnamic acids, which are known to promote stability in food (*12*); moreover,

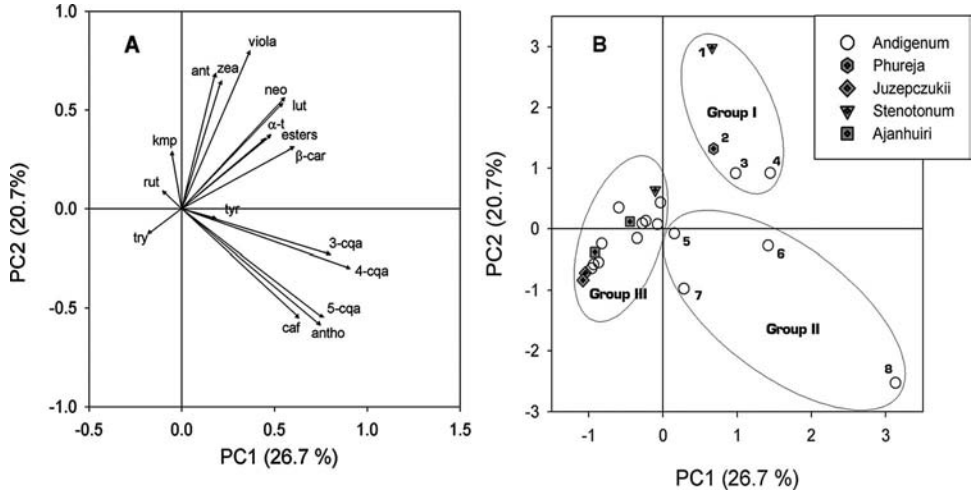


Figure 3. PCA resulting from the antioxidant profiling of 23 native Andean potato cultivars: (**A**) loading plot showing the relationships between variables; (**B**) score plot of the 23 potato genotypes. Each of the 23 observations is assigned to one of the three groups. Significance of numbers in panel **B** is defined in the text. Abbreviations: tyr, tyrosine; try, tryptophan; 3-cqa, neochlorogenic acid; 4-cqa, cryptochlorogenic acid; 5-cqa, chlorogenic acid; antho, total anthocyanins; caf, caffeic acid; rut, rutin; kmp, kaempferol-3-rutinoside; ant, antheraxanthin; zea, zeaxanthin; viola, violaxanthin; neo, neoxanthin; lut, lutein; α-t, α-tocopherol; esters, total esterified carotenoids; *β*-car, *β*-carotene.

the amount of anthocyanins found in our tubers, especially in 704429-Guincho Negra, is exceptionally high.

Principal Component Analysis. PCA was performed on the 23 potato genotypes, and 17 variables consisting of the measurements reported in **Tables 1** and **2** were included in the test. The first two principal components (PC) accounted for 47.4% of the total variance, PC1 (26.7%) and PC2 (20.7%) (**Figure 3**). As can be seen on the loading plot (**Figure 3A**), the different lipophilic variables (antheraxanthin, zeaxanthin, violaxanthin, neoxanthin, lutein, α-tocopherol, total esterified carotenoids, and *β*-carotene) showed similar vector directions, indicating a strong relationship between these molecules. Similarly, the different phenolic compounds (neochlorogenic acid, cryptochlorogenic acid, chlorogenic acid, total anthocyanins, and caffeic acid) appeared to be highly correlated between them. In contrast, orthogonality between these two groups of antioxidants can be observed in **Figure 3A**. This finding was previously reported with the total carotenoids and phenolics on 74 potato genotypes (*21*), supporting the hypothesis of independent control pathways between them. The directions of the vectors suggested that PC1 explains the variability in quantity, whereas PC2 rather explains the differences in quality. The short length of the vectors of tyrosine, tryptophan, rutin, and kaempferol-3-*O*-rutinoside indicates that these variables are correlated with principal components other than PC1 and PC2. These molecules were therefore not taken into account in this analysis. As indicated in **Figure 3B**, three different groups were observed. PC1 mainly discriminates the cultivars of group III from those of groups I and II, whereas PC2 mainly discriminates cultivars of group I from group II. Cultivars of group I (1, 702472-Amarilla del Centro; 2, 701570-Chaucha; 3, 704353-Puma; and 4, 703905-Huata Colorada) are mainly characterized by high proportions of carotenoids, whereas those of group II (5, 703750-Carganaca; 6, 702316-Pulu; 7, 702477-Yana Puma Maqui; and 8, 704429-Guincho Negra) are defined by high proportions of phenolic acids and anthocyanins. Besides, cultivars from group I are yellow-fleshed, whereas those from group II are purple-fleshed. Cultivars from group III are pigmented to a lesser extent and are characterized by their low or moderate occurrence of the different phenolic and carotenoid compounds. The patterns observed for the Phureja and Stenotonum groups demonstrate their high concentration in carotenoids, whereas the one for the genotypes from the Juzepczukii group shows their particularly low level in carotenoids. The very diverse antioxidant profiles found within the Andigenum group confirmed the high level of polymorphism of this group stressed in our previous study (*21*). Of particular interest are the cultivars from group II (from the Andigenum group only), which can be distinguished by their high anthocyanin content.

Conclusion. The main dietary antioxidant compounds present in Andean potato tubers were investigated from a nutritional perspective. In terms of quality, molecules identified in the native Andean cultivars were not different from the ones reported in the literature for their modern counterparts. Of great interest was yet the quantity of antioxidants recovered in distinct cultivars.

It is also noteworthy that this study was performed on raw unpeeled tubers that were stored for 4 months. The results reported here represent therefore the starting potential of the tubers, especially when they are discussed in terms of contribution to the dietary intake. The way the tubers will be processed before eating will indeed affect these values to various extents.

This study provides useful information concerning the potential of the native potato germplasm to contribute to the dietary antioxidant intake. In addition to the well-known high vitamin C content of potato in general, the great levels of α-tocopherol, *β*-carotene, zeaxanthin, chlorogenic acid, and petanin reported here in native potato cultivars offer a new opportunity to promote the nutritional quality of potato. These varieties could be exploited through direct use or through crop improvement. Purple-fleshed native species represent also an important source of health-promoting phytochemicals such as acylated anthocyanins.

ABBREVIATIONS USED

CQA, caffeoylquinic acid; CIP, Centro Internacional de la Papa; DAD, diode array detector; DW, dry weight; ESI, electron spray ionization; FLD, fluorescence detector; FW, fresh weight; HPLC, high-performance liquid chromatography; MS, mass spectrometry; PCA, principal component analysis; RNI, reference nutrient intake.

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