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Characterization and Quantitation of Anthocyanins and Other Phenolics in Native Andean Potatoes

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ABSTRACT: Andean potatoes are gaining popularity not only for their appealing colors and culinary uses but also for their potential higher content of polyphenolic compounds. The objective of this study was to identify potato varieties with increased phenolic content. This was achieved through characterization and quantitation of the phenolic composition in 20 varieties of native Andean potatoes from 4 different *Solanum* species with different colors. Major quantitative and qualitative differences among evaluated samples were more dependent on the coloration of the extracted sample rather than on the species. The most predominant anthocyanidins were petunidin-3-coumaroylrutinoside-5-glucoside and pelargonidin-3-coumaroylrutinoside-5-glucoside in purple and red potato extracts, respectively, while chlorogenic acid and its isomers were the main phenolic compund (43% of the total phenolic content). Our study suggested that the appropriate selection of native potatoes could provide new sources of polyphenolics with health promoting properties and natural pigments with increased stability for food applications.

KEYWORDS: *Andean tubers, potato, anthocyanins, polyphenols, chlorogenic acid*

INTRODUCTION

Potato is the fifth most produced crop worldwide after sugar cane, maize, wheat and rice with a production of >368 million tons in 2012.[1] Potatoes are a good source of dietary energy, carbohydrates, fiber, vitamin C, vitamin B1, niacin, vitamin B6 and minerals such as potassium, phosphorus and magnesium.[2] Besides basic nutrients, potatoes also provide a good source of phenolic compounds.

Correlation between ingesting phenolic compounds and improved health has been shown in epidemiological studies.[3−6] Phenolic compounds were shown to have antioxidant activity and other characteristics that could promote health. Among phenolics, anthocyanins are of special interest not only due to their health benefits but also due to their possible utilization as natural food pigments.[7,8]

The Andes are home to a great diversity of native potato species which have been traditionally consumed and solely grown in the Andes region by locals. Native Andean potato tubers show a wide variability in tuber shape, flesh and skin color, texture and flavor. This genetic diversity also generates substantial variability in nutritional contents.[2,9] However, very little is known about the chemical nature and identity of the bioactive compounds present in native Andean potatoes.[9]

The present study was designated to evaluate and characterize polyphenolics and anthocyanin content in native Andean potato varieties. This fits into a long-term objective of enhancing nutrition through diet and delivering health-enhancing components through stable crop commodities. For this study, the objective was to monitor and compare the phenolic content and profile of 20 different native Andean potato varieties from different species, which exhibited a variety of colors. The study included the measurement of the total phenolic, monomeric and polymeric anthocyanin content as well as the qualitative characterization of the anthocyanins and nonanthocyanin phenolics fraction. The hypothesis was that among the wide variety of native Andean potatoes, there are some that could represent important sources of phenolic components and could increase consumption of phytochemicals and antioxidants in the human diet.

MATERIALS AND METHODS

Chemicals. Reagent grade trifluoroacetic acid (TFA), certified ACS grade HCl (12N), LC−MS grade 99.9% methanol, 99.9% acetonitrile and water, certified ACS grade ethyl acetate and potassium hydroxide, HPLC grade 88% formic acid, 99.9% acetone and 99.9% chloroform were purchased from Fisher Scientific (Fair Lawn, NJ). Chlorogenic acid, caffeic acid, ferulic acid, *p*-coumaric acid and protocatechuic acid standards, reagent grade gallic acid, and Folin-Ciocalteau reagent were purchased from MP Biomedical (Aurora, OH). Sinapic acid standard (98%) was purchased from Sigma-Aldrich (St. Louis, MO). Chlorogenic acid isomers were prepared as described by Nagels.[10] Anthocyanidin standards were prepared from the acid hydrolysis of strawberries and grapes as described by Wrolstad and others.[11]

Plant Materials. A total of 20 samples (Table 1) of different colors (cream, yellow, brown, pink, red and purple) and species (*S. tuberosum*, *S. stenotonum*, *S. phureja* and *S. chaucha*) were used in this study. Potatoes were harvested by the International Potato Center (CIP), lyophilized and powdered with skin before shipping. Depending on the availability of the samples, between 150 and 500 g potato tubers were washed and cut into 1 cm^3 cubes. Cubes were then mixed and a random 100 g was weighed and placed in a polyethylene bag. Samples were immediately frozen at −20 °C and kept frozen until liophilized. Following the liophilization, native Andean potato samples

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Table 1. CIP Accession Number, Species Name, Lyophilized Sample Color Description and Water Activity of Analyzed Samples

CIP accession number	species name	lyophilized sample color	aw/°T
705534	*S. stenotomum* subsp. *stenotomum*	very bright purple with white spots	0.047/22.4 °C
703640	*S. tuberosum* subsp. *andigenum*	very bright purple with white spots	0.209/20.4 °C
706726	*S. tuberosum* subsp. *andigenum*	purple with black and white spots	<0.034/22.3 °C
704733	*S. tuberosum* subsp. *andigenum*	dark purple gray	0.085/22.1 °C
703862	*S. tuberosum* subsp. *andigenum*	purple cream with dark purple spots	0.072/20.9 °C
704133	*S. stenotomum* subsp. *stenotomum*	gray purple with purple spots	0.049/21.3 °C
702556	*S. stenotomum* subsp. *stenotomum*	purple pink	0.059/20.9 °C
706630	*S. tuberosum* subsp. *andigenum*	pink purple	<0.034/21.9 °C
700234	*S. tuberosum* subsp. *andigenum*	red purple with brown and white spots	0.044/21.7 °C
705841	*S. tuberosum* subsp. *andigenum*	red purple with brown and white spots	<0.034/21.1 °C
703752	*S. tuberosum* subsp. *andigenum*	red brown with brown spots	<0.034/22.6 °C
703695	*S. tuberosum* subsp. *andigenum*	brown	<0.034/21.2 °C
705820	*S. phureja*	cream with brown spots	<0.033/20.5 °C
704537	*S. chaucha*	cream white with dark spots	<0.034/21.3 °C
705946	*S. stenotomum subsp. stenotomum*	cream purple with dark spots	0.038/23.4 °C
705500	*S. stenotomum* subsp. *goniocalyx*	cream with brown spots	0.054/22.3 °C
702464	*S. stenotomum subsp. goniocalyx*	cream with brown spots	0.053/22.9 °C
703782	*S. stenotomum* subsp. *stenotomum*	yellow with brown spots	0.069/22.5 °C
706884	*S. stenotomum* subsp. *goniocalyx*	yellow with brown spots	<0.034/21.4 °C
704481	*S. stenotomum* subsp. *goniocalyx*	bright yellow with brown spots	<0.034/22.5 °C

were shipped by the CIP to our laboratory. Samples were kept under dark and at −40 °C until analysis. Lyophilization with skin caused the presence of skin pieces irregularly distributed in the sample. In some of the samples, skin had darker colors and highly contributed to the sample pigment content. During the weighing step, samples were taken from different parts of the containers to have a final representative sample as homogeneous as possible.

Extraction of Anthocyanins and Other Phenolics from Potato Samples. Lyophilized potato powder (~3 g) was blended with 45 mL of acidified aqueous acetone (20:80 v/v acidified with 0.1% HCL and 0.2% TFA) for 5 min with a tissuemiser homogenizer (Fisher Scientific, Fair Lawn, NJ) over a water/ice bath to reduce enzymatic activity. The mixture was centrifuged for 5 min at 3400 g (Centrific centrifuge Model 225; Fisher Scientific, Fair Lawn, NJ) and the supernatant was filtered through a Whatman #1 filter by vacuum suction using a Buchner funnel. The residual cake was reextracted with 25 mL of aqueous acetone (20:80 v/v acidified with 0.1% HCL and 0.2% TFA). Filtrates were combined and transferred to a separatory funnel and gently mixed with 2 volumes of chloroform. Samples were stored at 4 °C for 4 h in the dark. The aqueous phase (top layer) was separated and centrifuged for 7 min (3400 g). The aqueous supernatant was recovered and residual acetone/chloroform was removed using a Büchii Rotavapor (New Castle, DE) at 40 °C under vacuum. The remaining aqueous potato extract was then taken to 5 mL on a volumetric flask with acidified water (0.01% HCl). Extraction was done in triplicate for each sample.

Solid Phase Purification of Phenolic Fraction. The aqueous potato extract was purified using a Sep-pak C_{18} Vac solid cartridge (6 cm^3, 1 g sorbent; Waters Corp., Milford, MA). The C_{18} cartridge was activated with 10 mL of methanol and washed with 10 mL of acidified water (0.01% HCl). Potato extract (1 mL) was passed through the cartridge, anthocyanins and other phenolics were bound to the C_{18} cartridge; sugars and other polar compounds were removed with 10 mL of acidified water (0.01% HCl). The phenolic fraction was eluted with 10 mL of acidified methanol (0.01% HCl). The procedure was performed twice for every sample. Methanol was removed in a Büchii rotary evaporator at 40 °C under vacuum and the phenolic fraction was taken to 5 mL on a volumetric flask with acidified water (0.01% HCl). Purified phenolic extract was filtered (0.45 μm Whatman polypropylene filter) for further HPLC analysis.

Saponification of Anthocyanins. Aqueous potato extracts (1 mL) were saponified in a screw-cap test tube with 10 mL of 20% aqueous KOH for 10 min at room temperature in the dark as described by Durst and Wrolstad.[12] The solution was neutralized with the addition of 4 N HCl until a red color was seen and applied to an activated Sep-pak C_{18} Vac solid cartridge. Column was rinsed with 10 mL of acidified water (0.01% HCl) and liberated acid groups were removed with 10 mL of ethyl acetate. Saponified anthocyanin fraction was eluted with acidified methanol (0.01% HCl) and rotoevaporated at 40 °C under vacuum. The saponified fraction was taken to 5 mL on a volumetric flask with acidified water and filtered (0.45 μm Whatman polypropylene filter) for further HPLC analysis.

Acid Hydrolysis of Anthocyanins. Saponified anthocyanin extracts (3 mL) were hydrolyzed with 10 mL of 4 N HCl in a screw-cap test tube. The test tube was flushed with nitrogen gas, sealed and placed in a boiling water bath for 60 min in the dark and then cooled in an ice bath.[7] The solution was applied to an activated Sep-pak C_{18} Vac solid cartridge. The column was rinsed with 10 mL of acidified water (0.01% HCl) and the hydrolyzed anthocyanidin fraction was collected with acidified methanol (0.01% HCl) and rotoevaporated at 40 °C under vacuum. The hydrolyzed anthocyanidin fraction was taken to 2 mL on a volumetric flask with acidified water and filtered (0.45 μm Whatman polypropylene filter) for immediate HPLC analysis.

Monomeric and Polymeric Anthocyanin Content. Monomeric anthocyanin content was determined using the pH differential method as described by Giusti and Wrolstad.[13] Each aqueous potato extract was appropriately diluted using pH 1.0 and pH 4.5 (0.4 M sodium acetate) buffers. Solutions were allowed to equilibrate for 15 min in the dark. Absorbance was read on 1 cm path length disposable cuvettes at 510 nm (for red potato extracts) or 520 nm (for purple potato extracts) and 700 nm using a UV−visible Spectrophotometer 2450 (Shimadzu Scientific Instruments, Inc., Columbia, MD). Monomeric anthocyanin content (MAC) (mg/L) was calculated using the equation given by Giusti and Wrolstad.[13] Analyses were performed for each extraction replication (n = 3).

Polymeric anthocyanin content was also determined using the method described by Giusti and Wrolstad.[13] Using the dilution factors previously determined, an aliquote (1.85 mL) of diluted sample in acidified water (0.01% HCl) was prepared in each of two cuvettes (1 cm path length); then 0.15 mL of water was added to one of the cuvettes and 0.15 mL of potassium metabisulfate solution to the other and allowed to equilibrate for 15 min before reading at 420, 510 nm (for red potato extracts) or 520 nm (for purple potato extracts) and 700 nm using a UV−visible spectrophotometer 2450 (Shimadzu Scientific Instruments, Inc., Columbia, MD). Polymeric anthocyanin content was calculated using the equations given by Giusti and Wrolstad.[13] Analyses were performed for each extraction replication (n = 3).

Total Phenolics Quantitation. Total phenolics were measured using the microscale protocol for Folin-Ciocalteau colorimetry as described by Waterhouse.[14] Aliquotes (20 μL) of aqueous potato extract, a gallic acid calibration standard and a water blank were placed into separate 1 cm path length (2 mL) plastic cuvettes. Then 1.58 mL

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Table 2. Monomeric and Polymeric Anthocyanin and Total Phenolic Content in Potato Extracts[a]

CIP accession number	monomeric anthocyanins	% polymeric color	total phenolics mg GAE/100 g DW	% of total phenolics corresponding to monomeric anthocyanins
Section I: purple potato extracts				
705534	152.7 ± 22.3	20.0 ± 5.9	412.49 ± 40.95	37.02%
703640	145.2 ± 5.0	18.1 ± 13.5	510.20 ± 41.43	28.45%
706726	46.7 ± 3.8	20.2 ± 6.4	278.73 ± 24.00	16.77%
704733	41.1 ± 2.4	34.6 ± 2.5	379.20 ± 7.93	10.83%
703862	25.6 ± 0.9	28.8 ± 13.9	178.99 ± 11.71	14.28%
704133	16.8 ± 1.7	25.2 ± 9.9	162.19 ± 10.54	10.38%
Section II: red potato extracts				
702556	90.9 ± 8.5	12.4 ± 6.9	261.49 ± 18.63	34.76%
706630	88.7 ± 14.4	17.2 ± 4.9	337.71 ± 7.88	26.25%
700234	79.8 ± 3.5	20.5 ± 5.2	266.18 ± 7.67	29.99%
705841	75.6 ± 3.1	19.0 ± 7.6	304.17 ± 8.95	24.87%
703752	56.7 ± 3.6	24.8 ± 7.1	249.67 ± 24.24	22.72%
703695	54.6 ± 2.4	27.6 ± 8.0	241.93 ± 22.63	22.58%
705820	42.8 ± 2.4	13.9 ± 4.0	152.40 ± 14.86	28.10%
704537	38.1 ± 6.5	21.8 ± 7.0	153.36 ± 14.73	24.86%
705946	29.4 ± 2.9	34.0 ± 2.6	231.05 ± 22.00	12.71%
705500	25.9 ± 0.9	27.5 ± 10.2	199.75 ± 28.22	12.95%
702464	25.3 ± 1.0	26.2 ± 8.2	162.29 ± 9.88	15.57%
703782	8.2 ± 0.5	40.3 ± 1.1	159.15 ± 15.38	5.17%
Section III: yellow potato extracts				
706884	N/A	N/A	114.63 ± 10.75	N/A
704481	N/A	N/A	113.37 ± 6.36	N/A

[a] Section I: purple potato extracts (monomeric anthocyanin expressed as mg of cy-3-glu equivalents/100 g DW). Section II: red potato extracts (monomeric anthocyanin expressed as pg-3-glu equivalents/100 g DW). Section III: yellow potato extracts (Total phenolic content is expressed as mg of gallic acid equivalents (GAE) per 100 g of DW). Data are means ± SE (n = 3).

of water was added to each cuvette, followed by 100 μL of Folin-Ciocalteu reagent thoroughly mixed and incubated for 7 min. After incubation, 300 μL of sodium carbonate solution was added, mixed and allowed to incubate for 2 h at room temperature. Absorbance of samples, standards and blanks at 765 nm were measured on a UV−visible spectrophotometer 2450 (Shimadzu Scientific Instruments, Inc., Columbia, MD). Absorbance of the blank was subtracted from all readings and a calibration curve was created from the standards. Total phenolic content was calculated as gallic acid equivalents (GAE) based on the gallic acid calibration curve.

HPLC−MS Characterization of Samples. Samples were analyzed using a Shimadzu LCMS-2010 EV Liquid Chromatograph Mass Spectrometer (Shimadzu Scientific Instruments, Inc., Columbia, MD) equipped with LC-20AD pumps and SIL-20AC autosampler coupled with a SPDM20A photodiode array (PDA) detector and a single quadrupole electron spray ionization (ESI) mass spectrometer (MS) detector. Data was analyzed and processed using the LCMS Solutions software (Version 3, Shimadzu Scientific Instruments, Inc., Columbia, MD). Separation of anthocyanins and other phenolics in red extracts was achieved on a reverse phase Symmetry C_{18} column (3.5 um, 4.6 × 150 mm^2; Waters Corp., Milford, MA) and a Symmetry 2 micro guard column (4.6 × 22 mm^2; Waters Corp., Milford, MA). Chromatographic conditions were as follows: flow rate, 0.8 mL/min; mobile phase A, 4.5% formic acid in LCMS grade water; mobile phase B, LC−MS grade acetonitrile; gradient 0−5 min, 5% B; 5−20 min, 5−15% B; 20−30 min, 15% B; 30−40 min, 15−20% B; 40−45 min, 20−30% B; 45−48 min, 30−5% B and after each run 5 min was given to equilibrate the column to initial condition; injection volume varied according to the sample and ranged from 60 to 120 μL. Spectral data (250−700 nm) was collected during the whole run. Elution of total phenolics was monitored at wavelength 280 nm, cinnamic acids at 320 nm and anthocyanins at 510−520 nm.

Separation of anthocyanins and other phenolics in purple extracts was achieved using the following chromatographic conditions: flow rate: 0.8 mL/min; mobile phase: A, 10% formic acid in LCMS grade water; B, LC−MS grade acetonitrile; gradient: 0−5 min, 5% B; 5−16 min, 5- 13% B; 16−26 min, 13% B; 26−40 min, 13−20% B; 40−45 min, 20−30% B; 45−48 min, 30−5% B and after each run 5 min was given to equilibrate the column to initial condition; injection volume varied according to the sample and ranged from 60 to 120 μL. Spectral data (250−700 nm) was collected during the whole run. Elution of anthocyanins was monitored at wavelength 510−520 nm. Separation of anthocyanidins in hydrolyzed samples was achieved using the following chromatographic conditions: flow rate, 0.8 mL/min; mobile phase A, 4.5% formic acid in LC−MS grade water; mobile phase B, LC−MS grade acetonitrile; gradient 0−30 min, 5−35% B; 30−35 min, 35% B; 35−38 min, 35−5% B; 38−41 min, 5% B; injection volume: 120 μL. Spectral data (250−700 nm) was collected during the whole run. Elution of anthocyanins was monitored at wavelength 510−520 nm.

A 0.2 mL/min flow was diverted to the MS. Mass spectra were obtained under positive ion condition using total ion scan (SCAN) (from m/z 150 to 1300) and selective ion monitoring (SIM) modes. Eight channels including m/z 181 (caffeic acid), m/z 271 (pelargonidin), m/z 287 (cyanidin), m/z 301 (peonidin), m/z 303 (delphinidin), m/z 317 (petunidin), m/z 331 (malvidin) and m/z 355 (chlorogenic acid), were monitored in the SIM mode to detect the 6 common anthocyanin aglycone fragments and two common phenolic acids.

RESULTS AND DISCUSSION

Sample Characteristics. Detailed information about the species used in the study including water activity (aw) values of the lyophilized samples measured prior to sample extraction is shown in Table 1. All samples had aw lower than 0.086 (at ∼21 °C) except for sample 703640 which had aw of 0.209. The average moisture content in the samples was 2.07%, with sample 703640 being the only variety with moisture content higher than 4% (∼6%) (moisture data is not shown). In general the lyophilization process removed most of the moisture from the potato samples.

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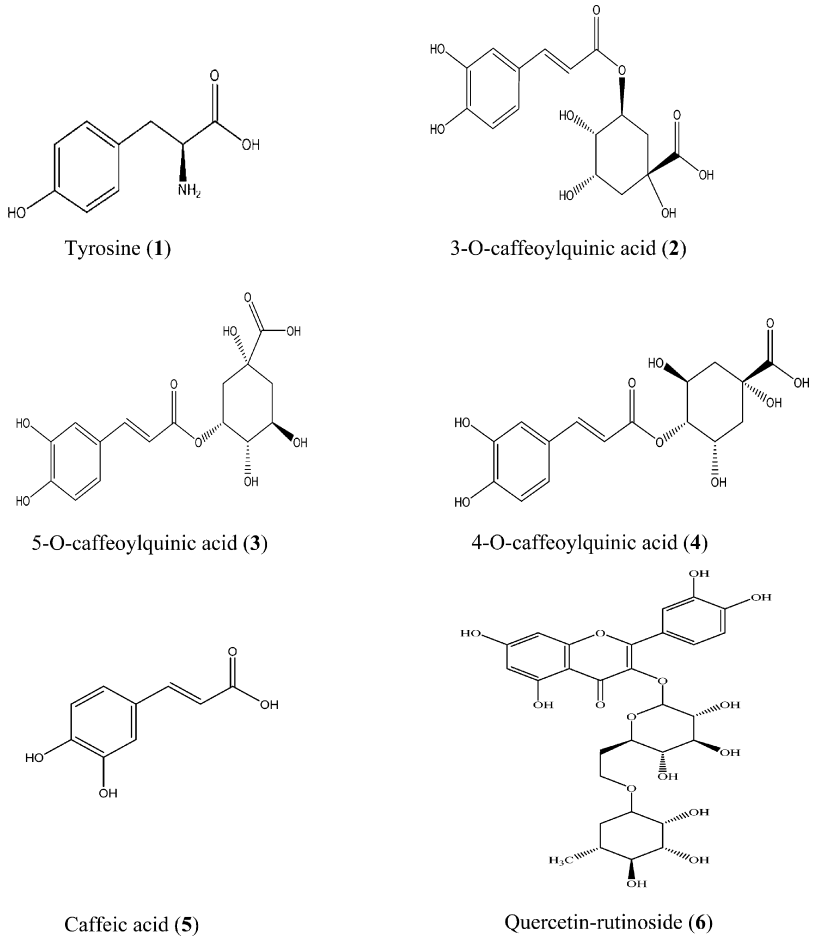

Figure 1. Chemical structures of the major phenolic compounds identified in Andean potatoes by HPLC PDA/MS. *Compound numbers refer to the peak numbers given in Figure 2.

Table 3. List of Phenolic Compounds Identified in Andean Potatoes by HPLC PDA/MS

peak no[a]	retention time (min)[a]	M^+ (m/z)	peak assignment	reference compounds
1	3.3	182	tyrosine	tentatively identified
2	7.5	355	3-*O*-caffeoylquinic acid	chlorogenic acid isomers[b]
3	12.8	355	5-*O*-caffeoylquinic acid	chlorogenic acid isomers[b]
4	14.2	355	4-*O*-caffeoylquinic acid	chlorogenic acid isomers[b]
5	15.0	181	caffeic acid	caffeic acid standard
6	26.5	611	quercetin-rutinoside derivative	tentatively identified

[a]Compound numbers and retention times refer to the numbers given in Figure 1. [b]Chlorogenic acid isomers were prepared as described by Nagels.[10]

Improvement of the Extraction. Initially, phenolic fraction of the potatoes was extracted following the procedure described by Shiroma-Kian and others.[15] However, during extraction of selected potato samples, some browning occurred and anthocyanin coloration was lost suggesting enzymatic degradation. According to Francis,[16] anthocyanins can be degraded by a number of enzymes classified as glycosidases (anthocyanases), polyphenoloxidases (PPO) and peroxidases. These enzymes are found in plant tissue and naturally occur in potatoes. Glycosidases act directly on anthocyanins hydrolyzing the pigments to sugars and anthocyanidins (aglycone).[16,17] Anthocyanidins are very unstable and degrade to colorless derivatives or condense with other phenolics to form polymeric colored compounds.[15] The action of PPO and peroxidases on anthocyanins is indirect.[11] For example, PPO oxidizes *o*-diphenols (phenolic acids, e.g., chlorogenic acid) to *o*-benzoquinone which in the presence of oxygen reacts with the anthocyanins by a nonenzymatic mechanism to form oxidized anthocyanins and degradation products.[17] In order to minimize pigment degradation, the extraction process of anthocyanins and other phenolics from potato samples was modified. The percent of acid and acetone in the aqueous acetone solution was increased and the time for the homogenization was reduced. The pH of a solution can have a marked effect on enzymatic activity. Enzymes that degrade anthocyanins have an optimum pH greater than 4.[18] The pH of 70% aqueous acetone acidified with 0.01% HCl was lower than pH 3. However, after homogenization with the potato powder, the pH of the solution was higher than pH 5. This suggested that the potato powders had high buffering capacity and were increasing the pH of the solution. In order to obtain final pH values lower than 3.5 after homogenization, the acidity of the aqueous acetone solution was increased with additional HCl and trifluoroacetic acid (TFA). A combination of 0.1% HCl and 0.2% TFA resulted on a final extract with a pH between 3−3.5 that minimized enzymatic activity and favored pigment stability.

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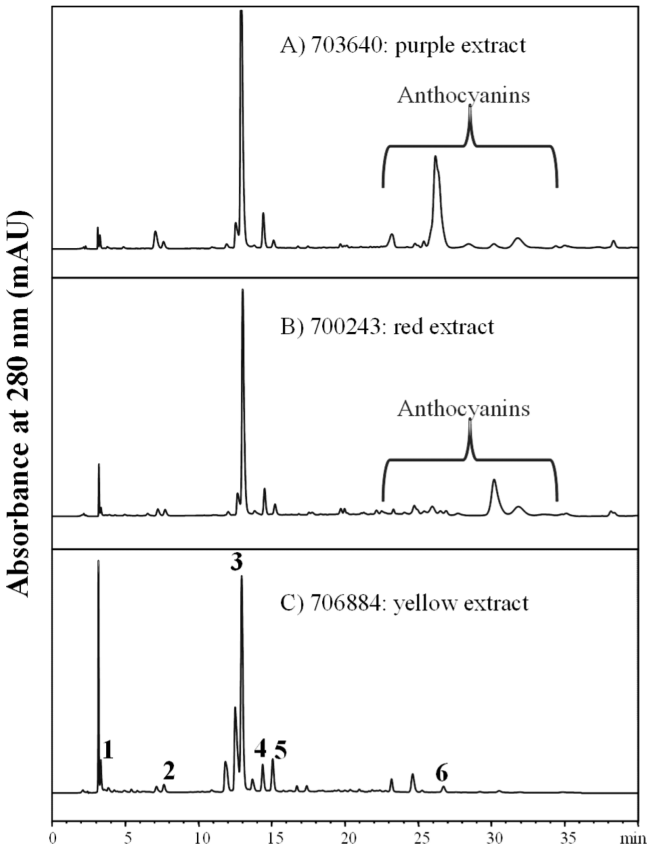

Figure 2. HPLC chromatogram at 280 nm of phenolics compounds in native Andean potatoes. *Compound 1 and 6 were tentatively identified.

Table 4. Relative Phenolic Composition of Potato Samples (% of Total Peak Area at 280 nm)[a]

CIP accession number	1[b]	2	3	4	5
705534	0.15	1.22	25.03	3.05	1.81
703640	0.77	0.95	33.76	3.88	1.12
706726	0.74	2.00	38.52	5.29	2.39
704733	0.48	0.66	39.84	3.87	5.19
703862	0.93	1.55	33.78	4.10	3.23
704133	2.73	2.43	31.73	5.90	3.33
702556	0.37	1.27	35.17	3.98	2.94
706630	0.51	3.51	38.21	8.13	1.78
700234	0.75	1.27	40.95	4.11	1.85
705841	0.80	3.63	41.83	9.75	1.70
703752	0.40	0.85	20.79	4.66	1.88
703695	1.46	0.96	30.23	4.51	1.53
705820	1.39	1.68	24.97	5.44	1.11
704537	1.13	1.24	34.93	2.92	3.67
705946	1.77	1.72	45.96	7.36	3.29
705500	1.25	4.53	45.39	8.60	3.15
702464	0.28	2.56	51.52	9.24	3.71
703782	1.46	2.21	33.34	5.16	7.85
706884	3.28	1.55	36.90	4.21	5.55
704481	1.17	1.30	31.48	6.83	8.42

[a]Compound numbers refer to the numbers given in Table 3. [b]Tentative identification.

Another important consideration was that the extracted samples were going to be concentrated after the chloroform partitioning. High concentrations of acid could hydrolyze the phenolic compounds and anthocyanins in the sample. TFA was chosen to acidify the solution since, even though it is a strong acid, it is also more volatile than HCl and could evaporate during the concentration of the sample.

To better protect the integrity of the sample and minimize enzymatic activity, higher concentrations of acetone could be used during extraction. A small trial with different concentrations of acetone in the extraction solution was performed using 70, 80, 90 and 100% of acidified acetone. The solution with 80% aqueous acidified acetone was the most efficient and was chosen as the extracting solvent for this research.

The homogenization process was also improved using a tissuemiser homogenizer and reducing the mixing time from 10 to 5 min. The previous procedure called for homogenization on a rotamixer took longer time to extract the phenolics from the samples. In addition to decreasing the time involved in the extraction, changing to a tissumizer allowed us to incorporate a water/ice bath to reduce temperature and diminish any residual enzymatic activity. Usually when chloroform partitioning is performed in a sample, two layers are formed. The top aqueous layer contains the phenolic fraction in water and the bottom layer contains the mixture of chloroform and acetone. In our extraction procedure, an additional layer was formed between the chloroform/acetone and the aqueous layers. To separate it from the phenolic (aqueous) fraction, a centrifugation step was applied.

Total Phenolic Quantitation. Phenolic content of potato samples ranged from 113.37 to 510.20 mg of gallic acid equivalents (GAE) per 100 g of DW (Table 2). From the samples evaluated, potatoes from the species *S. tuberosum* subsp. *andigenum* and *S. Stenotomum* subsp. *stenotomum* had higher levels of phenolic compounds than the other species analyzed. Since the majority of the analyzed samples belonged to the *S. tuberosum* subsp. *andigenum* and *S. Stenotomum* subsp. *stenotomum* species (13 out of 20 samples), it can not be concluded as these species will always have higher phenolic content. More research needs to be done including more samples of different species to be able to determine if indeed samples of these two species have overall higher phenolic content than samples of other species. The sample with the highest total phenolic content ($p < 0.001$) was 703640, a *S. tuberosum* subsp. *andigenum* that exhibited a dark purple coloration in the extract with 510.2 mg of GAE/100 g DW, followed by sample 705534, a *S. stenotomum* subsp. *stenotomum*, dark purple extract and 704733, a *S. tuberosum* subsp. *andigenum*, a purple extract with 412.49 and 379.2 mg of GAE/100 g DW, respectively. Samples 706884 and 704481 both belonging to the specie *S. Stenotomum* subsp. *goniocalyx* produced yellow extracts and did not contain monomeric anthocyanins. These two samples had the lowest total phenolic content with 114.63 mg and 113.37 mg of GAE per 100 g DW, respectively. Phenolic content of the potato samples evaluated seemed to be more dependent on the coloration of the samples than on the specie, with higher phenolic content being obtained from samples exhibiting more intense colors. The values found in this research were higher than those reported by Im and others[19] in 24 regular American potatoes (including Yucon gold, Russet, red and white potatoes) bought in a local store in Berkely, California finding 4.09 mg to 281 mg of total phenolics per 100 g of DW. Only one variety studied by the authors gave higher values of phenolics, which was a Peruvian purple potato with 757 mg of total phenolics per 100g of DW.

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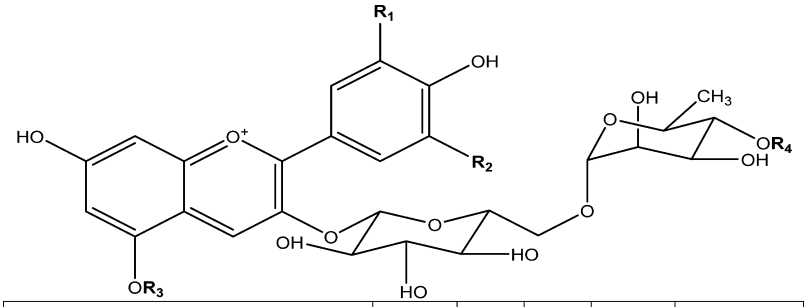

Name of Anthocyanin	Peak No	R_1	R_2	R_3	R_4
Pelargonidin-3-rutinoside-5-glucoside	7	H	H	Glucose	-
Pelargonidin-3-rutinoside	8	H	H	-	-
Pelargonidin-3-caffeoylrutinoside-5-glucoside	9	H	H	Glucose	Caffeic acid
Peonidin-3-caffeoylrutinoside-5-glucoside	10	OCH_3	H	Glucose	Caffeic acid
Cyanidin-3-coumaroylrutinoside-5-glucoside	11	OH	H	Glucose	Coumaric acid
Petunidin-3-coumaroylrutinoside-5-glucoside	11[I]	OCH_3	OH	Glucose	Coumaric acid
Pelargonidin-3-coumaroylrutinoside-5-glucoside	12	H	H	Glucose	Coumaric acid
Peonidin-3-coumaroylrutinoside-5-glucoside	13	OCH_3	H	Glucose	Coumaric acid
Pelargonidin-3-feruloylrutinoside-5-glucoside	14	H	H	Glucose	Ferulic acid
Peonidin-3-feruloylrutinoside-5-glucoside	15	OCH_3	H	Glucose	Ferulic acid
Petunidin-3-caffeoylrutinoside-5-glucoside	16	OCH_3	OH	Glucose	Caffeic acid
Petunidin-3-feruloylrutinoside-5-glucoside	17	OCH_3	OH	Glucose	Ferulic acid
Malvidin-3-coumaroylrutinoside-5-glucoside	18	OCH_3	OCH_3	Glucose	Coumaric acid

Figure 3. Chemical structures of major anthocyanins identified in red and purple potato extracts by HPLC PDA/MS. *Compound numbers refer to the peak numbers given in Figure 4.

Monomeric and Polymeric Anthocyanin Content. Based on the anthocyanin composition, colored samples have a maximum absorbance (λ_{max}) at different wavelengths. Most of the colored potato samples analyzed had their λ_{max} either at 520 nm (purple extracts) or at 510 nm (red extracts). Purple extracts with highest absorbance at 520 nm were quantitated as cy-3-glu equivalents (Table 2 Section I) while red extracts with λ_{max} at 510 nm were quantitated as pg-3-glu equivalents (Table 2 Section II).

The monomeric anthocyanin content of the different red and purple potato varieties ranged from 8.2 to 152.7 mg/100 g DW. The cultivars with the lowest monomeric anthocyanin content were 703782, a *S. stenotomum* subsp. *stenotomum* that exhibited a light purple extract and 704133, a *S. stenotomum* subsp. *Stenotomum* exhibiting a light red extract with 8.2 and 16.8 mg/100 DW, respectively. The varieties with highest pigment content were 705534, a *S. stenotomum* subsp. *stenotomum*, dark purple extract, and 703640, a *S. tuberosum* subsp. *andigenum*, dark purple extract, with more than 145 mg cy-3-glu equivalents/100 g DW. These two samples also had high content of total phenolics. Anthocyanin content of the potato samples evaluated were more related to the coloration of the samples than on the specie, since samples with more intense colors showed higher anthocyanin content. Higher monomeric anthocyanin content has been reported for other purple potato cultivars such as Urenika with average contents 507.8 and 183.6 mg/100 g FW in the skin and flesh, respectively.[20] Rodriguez-Saona and others[7] also reported higher contents of monomeric anthocyanins (>35 mg/100 g FW) in two different red potato selections. Similarly, Campos and others[9] found higher quantities of total anthocyanin content in fresh native Andean potato tubers (8 mg to 80 mg per 100 g of fresh tuber). There might be the potential to develop a red and purple potato clones through potato breeding studies with pigment content similar to those of Urenika.[7]

In the analyzed samples, the percentage of polymeric color ranged between 12.4% and 40.3% (Table 2) with an average polymeric color of 24%. The sample with the highest percentage of polymeric color was 703782 (*S. stenotomum* subsp. *stenotomum*, red extract), which was found to contain the lowest monomeric anthocyanin content as well. Polymeric color in the potato samples could be attributed to polymerization reactions during processing/lyophilization and over storage as well as some possible residual enzymatic activity during extraction.

Anthocyanin content as a percentage of the total phenolics ranged from 5.17% to 37.02% with an average of 20% of the total phenolics content (Table 2). With the exception of sample 704733, a *S. tuberosum* subsp. *andigenum* that produced a purple extract, total phenolic and anthocyanin content showed linear correlation (R^2 = 0.841, p-value = 0.0001). The reason for sample 704733 being an outlier was the high content of phenolic compounds (379.2 mg of GAE per 100 g of DW) and low content of monomeric anthocyanins (41.1 mg of cy-3-glu

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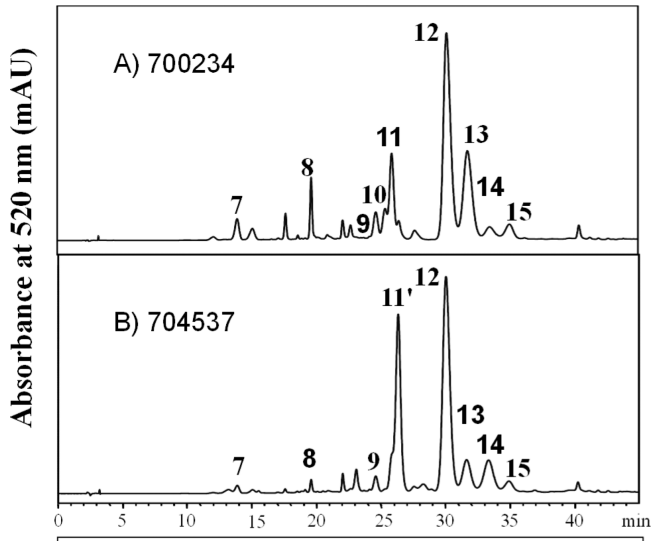
Table 5. List of Major Anthocyanins Identified in Red and Purple Potato Extracts by HPLC PDA/MS

peak no[a]	retention time (min)[a]	M^+ (m/z)	fragment ions	peak assignment[b]
7	13.4	741	271	pelargonidin-3-rutinoside-5-glucoside
8	19.5	579	271	pelargonidin-3-rutinoside
9	24.3	903	271	pelargonidin-3-caffeoylrutinoside-5-glucoside
10	25.1	933	301	peonidin-3-caffeoylrutinoside-5-glucoside
11	25.6	903	287	cyanidin-3-coumaroylrutinoside-5-glucoside
11[I]	26.3	933	317	petunidin-3-coumaroylrutinoside-5-glucoside
12	29.5	887	271	pelargonidin-3-coumaroylrutinoside-5-glucoside
13	31.1	917	301	peonidin-3-coumaroylrutinoside-5-glucoside
14	32.9	917	271	pelargonidin-3-feruloylrutinoside-5-glucoside
15	34.4	947	301	peonidin-3-feruloylrutinoside-5-glucoside
16	19.6	949	317	petunidin-3-caffeoylrutinoside-5-glucoside
17	27.3	963	317	petunidin-3-feruloylrutinoside-5-glucoside
18	31.9	948	331	malvidin-3-coumaroylrutinoside-5-glucoside

[a]Compound numbers and retention times refer to the numbers given in Figures 4 and 5. [b]All anthocyanins were identified based on the combined information on UV−vis, molecular ion, mass fragments and retention times of the anthocyanin and the anthocyanidins. Anthocyanidin identification was made using the acid hydrolysis of strawberry (for pelargonidin) and grape (for delphinidin, cyanidin, peonidin, petunidin and malvidin as references).



Code	7	8	9	10	11	11'	12	13	14	15
700234	2.3	4.1	3.3	3.4	10.1	ND	38.7	20.6	3.1	3.3
702464	3.4	1.2	2.5	ND	0.5	ND	69.2	1.7	14.6	1.5
702556	3.0	4.0	3.4	1.4	3.3	ND	53.5	10.7	9.5	3.8
703695	4.2	1.7	6.2	ND	2.0	ND	68.3	2.2	8.5	ND
703752	4.1	1.2	7.3	ND	2.9	ND	58.8	2.0	17.8	ND
703782	5.7	4.3	3.2	ND	4.2	ND	45.8	9.3	11.3	4.7
704537	0.9	0.7	1.7	ND	ND	28.8	43.5	7.6	8.6	2.3
705500	6.2	3.5	2.8	ND	2.0	ND	64.5	3.8	8.9	0.9
705820	11.3	15.4	4.0	ND	ND	ND	51.0	1.5	5.9	0.6
705841	5.9	6.8	3.0	1.3	7.1	ND	48.7	10.9	3.6	2.0
705946	4.4	10.6	4.4	ND	ND	ND	63.7	2.2	4.1	NQ
706630	6.3	6.8	3.2	1.5	5.9	ND	49.8	9.7	4.0	2.3

Figure 4. HPLC chromatograms at 520 nm for major anthocyanins of red potato extracts and their peak areas (% of total peak area at 520 nm). ND: not detected. Difference from 100 is due to the presence of minor peaks, each accounting for less than 2% of total area. *All anthocyanins were tentatively identified.

equivalents per 100 g of DW) it contained. This finding shows that even though the presence of anthocyanins increases the overall phenolic content, there might be potato varieties with high phenolic content that show little or no anthocyanin content.

Phenolic Characterization. Potato phenolics were characterized by monitoring the elution of compounds at 280 nm. Key structures for phenolics addressed are illustrated in Figure 1 and further detalied in Table 3. Three chromatograms representing purple, red and yellow extracts can be seen in Figure 2. Similar phenolic profiles were obtained for all potato samples besides the differences in the anthocyanin fractions (Figure 2).

The presence of chlorogenic, caffeic, *p*-coumaric, ferulic, sinapic, gallic and protocatechuic acid have been previously reported in potatoes.[7,20−22] Therefore, cocktails with these phenolic acid standards were injected in the HPLC−MS with the same separation method used for the potato samples. From the standards used, only chlorogenic and caffeic acid were identified among the phenolic acids in the samples. Chlorogenic acid isomers were prepared and injected in the HPLC−MS following the same method used for the potato samples and other standards. During the isomerization procedure, caffeic acid was also formed and eluted after the three isomers.

Phenolic identification was made based on UV, mass spectra and retention time comparisons to those of pure standards available. In addition to the anthocyanin peaks, 13 major phenolic peaks were found in the potato samples (Figure 2). From those peaks four compounds (**2**, **3**, **4** and **5**) have been clearly identified and two others (**1** and **6**) have been tentatively identified.

Relative proportions of the phenolic compounds (% of total peak area at 280 nm) identified were reported in Table 4. Proportions of the compound **6** (tentatively identified) were not included in Table 4 due to difficulty of quantitation since the compound coeluted with an anthocyanin peak. In average, **2**, **3** and **4** represented 43% of the total phenolic content. However, this value was lower than the 70% of total phenolic area reported by Rodriguez-Saona and others[7] in red potatoes and up to 90% of the total phenolic compounds in potato tubers analyzed by Malmberg and Theander.[23]

Anthocyanin Characterization. Anthocyanin profiles obtained were very similar among all samples within the same color (purple and red extracts). However, clear differences between anthocyanin profiles of purple and red samples were noticed from the HPLC chromatograms at 520 nm. Purple extracts had fewer peaks than the red extracts and in general, most predominant peaks in purple extracts eluted before the predominant peaks in red extracts. Due to their different profiles, both groups were characterized individually. To facilitate the identification of the anthocyanins, samples were saponified and hydrolyzed and analyzed through HPLC−MS. Anthocyanidin identification was made based on UV−vis and mass spectra and retention time comparisons to those of anthocyanidin standards obtained from the acid hydrolysis of

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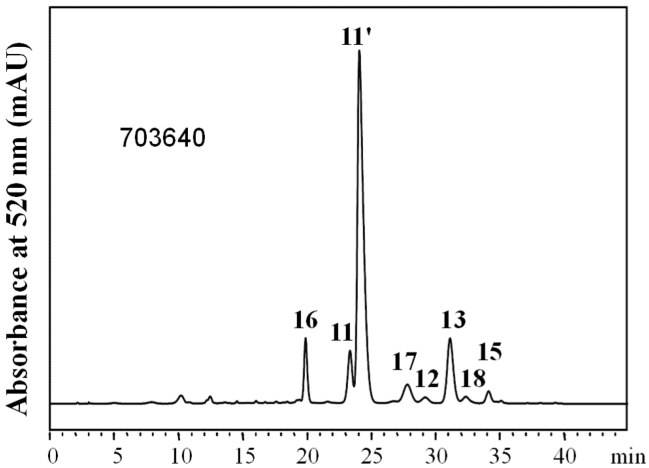

Code	16	11	11'	17	12	13	18	15
703640	6.1	7.1	63.6	4.5	1.3	11.9	1.3	1.5
703862	3.5	4.6	57.0	3.6	10.1	11.0	4.8	2.2
704133	4.0	6.3	61.6	3.0	1.2	15.9	3.0	2.0
704733	4.8	7.6	62.5	3.4	ND	15.9	2.4	1.3
705534	4.3	4.6	68.4	3.0	0.5	12.6	3.0	1.6
706726	5.3	7.4	65.7	2.8	ND	12.0	2.1	1.0

Figure 5. HPLC chromatogram at 520 nm for major anthocyanins in purple potato extract and their peak areas (% of total peal area at 520 nm). ND: not detected. Difference from 100 is due to the presence of minor peaks, each accounting for less than 2% of total area. *All anthocyanins were tentatively identified.

strawberry (pelargonidin) and grape (delphinidin, cyanidin, peonidin, petunidin and malvidin). Key structures of anthocyanins identified are illustrated in Figure 3 and further detailed in Table 5.

Red Potato Extracts. Three major anthocyanidins (cyanidin, pelargonidin and peonidin) were identified in red potato extracts. Among the saponified samples, four major peaks were identified based on UV−vis and mass spectra as cyanidin-3-rutinoside-5-glucoside, pelargonidin-3-rutinoside-5-glucoside, peonidin-3-rutinoside-5-glucoside, pelargonidin-3-rutinoside with pelargonidin-3-rutinoside-5-glucoside was being the most predominant peak in the saponified samples.

Seven of the nine anthocyanin major peaks in red potato extracts (Figure 4) were acylated with a cinnamic acid. Three different cinnamic acids were found acylating the anthocyanins in red potato extract: caffeic, *p*-coumaric and ferulic acid. Nine major anthocyanin peaks were identified based on UV−vis and mass spectra. Pelargonidin-3-*p*-coumaroyl-5-glucoside was the most predominant peak in red potato extracts after saponification which was in accordance with the findings of Rodriguez-Saona and others.[7] Five major peaks were pelargonidin derivatives: two nonacylated (compounds **7** and **8**) and three acylated (compounds **9**, **12** and **14**), while three pigments were acylated peonidin derivatives (compounds **10**, **13** and **15**) and only one major pigment was identified as acylated cyanidin derivative (compound **11**). Sample 704537, a *S. Chaucha specie*, presented slight differences in anthocyanin profile and therefore it was characterized individually. The main difference between sample 704537 and the other red potato extracts was the presence of compound $\mathbf{11}^{\mathbf{I}}$ (Figure 4).

Relative proportions (% of total peak area at 520 nm) of the anthocyanin compounds previously identified were also shown in Figure 4. In average, compound **12** accounted for 54.6% of the total anthocyanin content. However, this value was lower than the range between 56.7 and 83.2% of total area reported by Rodriguez-Saona and others[7] in red potatoes.

Purple Potato Extracts. Five major anthocyanidins (cyanidin, petunidin, pelargonidin, peonidin and malvidin) were identified in purple potato extracts. From the six common anthocyanidins found in nature, only delphinidin was not present in purple potato extracts. Petunidin and peonidin were the most predominant anthocyanidins in purple potato extracts.

Four major peaks were found in the saponified samples of purple potato extracts. The peaks were identified based on UV−vis and mass spectra as the 3-rutinoside-5-glycoside derivatives of the five anthocyanidins found in the hydrolyzed samples. According to order of their elution, they were: cyanidin-3-rutinoside-5-glucoside, petunidin-3-rutinoside-5-glucoside, pelargonidin-3-rutinoside-5-glucoside and peonidin-3-rutinoside-5-glucoside, with petunidin and peonidin glycosides being the most predominant peaks in the saponified samples.

The separation and identification of the acylated anthocyanins in purple potato extracts was more challenging since some of the peaks coeluted making their identification more difficult. Accordingly, the HPLC separation method was improved by changing the aqueous solvent concentration to 10% formic acid and slightly modifying the solvent gradient. Even though fractioning of the molecules and MS data collection was better with lower acid concentrations, better separation of the compounds in the C_{18} column was achieved with higher acidity. The same three acylating groups (caffeic, p-coumaric and ferulic) identified in red potato extracts were also found in the purple potato extracts. Eight major acylated anthocyanin peaks (Figure 5) were identified in purple potato extracts: **11**, $\mathbf{11}^{\mathbf{I}}$, **12**, **13**, **15**, **16**, **17**, **18**. Petunidin and peonidin acylated derivatives were the most predominant peaks in purple potato extracts.

Relative proportions (% of total peak area at 520 nm) of the anthocyanin compounds in purple potato extracts were also shown in Figure 5. In average, compound $\mathbf{11}^{\mathbf{I}}$ accounted for 63.2% of the total anthocyanin content.

In conclusion, Andean potato samples from different species showed great variability in polyphenolic and anthocyanin content. Major quantitative and qualitative differences among evaluated samples seemed to be more dependent on the coloration of the sample after extraction rather than on the specie. Potato varieties with high anthocyanin content could be used as sources of natural pigments with increased stability for food applications. Due to the high consumption levels of potatoes around the world, selected native Andean potatoes could be be introduced into the market to deliver significant amounts of phenolic compounds with potential health promoting properties into the human diet.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

CIP, international potato center; DW, dry weight; ESI, electron spray ionization; GAE, gallic acid equivalents; HPLC, high-pressure liquid chromatography; MS, mass spectroscopy; PDA,

photodiode array; PPO, polyphenol oxidase; SIM, selective ion monitoring; TFA, trifluoroacetic acid

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