

ORIGINAL RESEARCH

Relating genes in the biosynthesis of the polyphenol composition of Andean colored potato collection

Leslie Tejada^{1,2}, Juan Antonio Alvarado¹, Magdalena Dębiec¹, José Mauricio Peñarrieta¹, Oscar Cárdenas¹, María Teresa Alvarez³, Aakash Chawade⁴, Lars Nilsson² & Björn Bergenståhl²

¹School of Chemistry, Faculty of Pure and Natural Sciences, San Andrés University, P.O. Box 303, La Paz, Bolivia

²Food Colloids Group, Department of Food Technology, Engineering and Nutrition, Lund University, P.O. Box 124, S-221 00 Lund, Sweden

³Drug Research Institute and Biochemical, Faculty of Pharmaceutical and Biochemical Sciences, San Andrés University, P.O. Box 303, La Paz, Bolivia

⁴Department of Immunotechnology, Lund University, BMC D13, SE-22184 Lund, Sweden

Keywords

Ans and *stan* genes, anthocyanidins, antioxidant capacity, polyphenolic compounds and purple potatoes

Correspondence

Leslie Tejada, School of Chemistry, San Andrés University, P.O. Box 303, La Paz, Bolivia.
Tel: +591 2 2612825; Fax: +591 2 2772269;
E-mail: lktejeda@umsa.bo

Funding Information

Swedish International Development Agency (SIDA), which supported this thesis as part of the project "Antioxidants in Bolivian Food" at Mayor de San Andres University, La Paz, Bolivia.

Received: 10 May 2013; Revised: 15 August 2013; Accepted: 27 August 2013

Food Science & Nutrition 2014; 2(1): 46–57

doi: 10.1002/fsn3.69

Abstract

The objective of this study was to evaluate total antioxidant capacity (TAC), total phenolic content (TPH), and the identification of anthocyanidin and polyphenolic compounds in 13 colored potatoes collected from the Andean region of Bolivia, and understand how the chemical composition correlated with the botanical classification and molecular characterization of genes, *ans* (anthocyanidin synthase) and *stan1* (*Solanum tuberosum* anthocyanidin synthase), associated with the synthesis of anthocyanidins. The results show the existence of a limited correlation between botanical classification, based on morphological identification and polyphenol composition. No association between genetic grouping of the *ans* and *stan* genes and botanical classification was found. However, it was possible to identify a correlation between the *ans* gene clades and the levels of anthocyanidins as well as of other polyphenols. Thus, this result confirms the concept that potato color can be used in the search for high polyphenol potato cultivars.

Introduction

Potatoes accumulate a great variety of secondary metabolites, including polyphenolic and many other phytochemical compounds, as a protection against the adverse effects of solar radiation, and injury of herbivorous insects or pathogens such as bacteria or fungi (Del Mar Verde Méndez et al. 2004; Brown 2005). Polyphenolic compounds are able to form stable radicals and thereby act as radical scavengers and antioxidants. As antioxidants, polyphenols are expected to have potentially positive health

benefits (Del Mar Verde Méndez et al. 2004). Anthocyanidins is a group of polyphenols able to create red, purple, blue, or orange colors (Brown 2005).

Colored tubers contained mostly pelargonidin, peonidin, petunidin, malvidin, cyanidin, and delphinidin (Lewis et al. 1998; Alcalde-Eon et al. 2004; Hamouz et al. 2011; Lachman et al. 2012). Tuber parenchyma tissue also contained chlorogenic acid and other phenolic acids, plus low concentration of flavonoids. Determinations of polyphenols in periderm tissue have shown higher level of chlorogenic acid, moderate amounts of protocatechuic

acid, caffeic acid, vanillic acid, and sinapic acid, and low concentrations of gallic acid, syringic acid, catechin, p-coumaric acid, ferulic acid, salicylic acid, and cinnamic acid, where chlorogenic acid constitutes ~90% of the total phenolic content (Friedman 1997; Lewis et al. 1998; Del Mar Verde Méndez et al. 2004; Preedy et al. 2011).

Anthocyanidin biosynthesis is one of the most studied plant secondary metabolite pathways. There are many genes responsible for enzyme synthesis. For almost every biosynthetic step the corresponding enzymes have been isolated and a considerable body of knowledge is available on the mechanisms that regulate their expression in the plant cell (Deroles 2009). Two classes of genes are

required for anthocyanidin biosynthesis – the structural genes, for example, *ans* and *chi* that code for the enzymes that are directly involved in the production of anthocyanidin and the regulatory genes, for example, *stan1* and *stan2*, that control the transcription of structural genes (Al-Sane et al. 2011). The pathway for the anthocyanidin biosynthesis is shown in Figure 1.

In the current study, the chemical composition in terms of classes such as total antioxidant activity (TAC), total phenolic compounds (TPH) was characterized, and individual polyphenolic compounds were identified and quantified by high-performance liquid chromatography (HPLC). Molecular analysis of genes involved in the

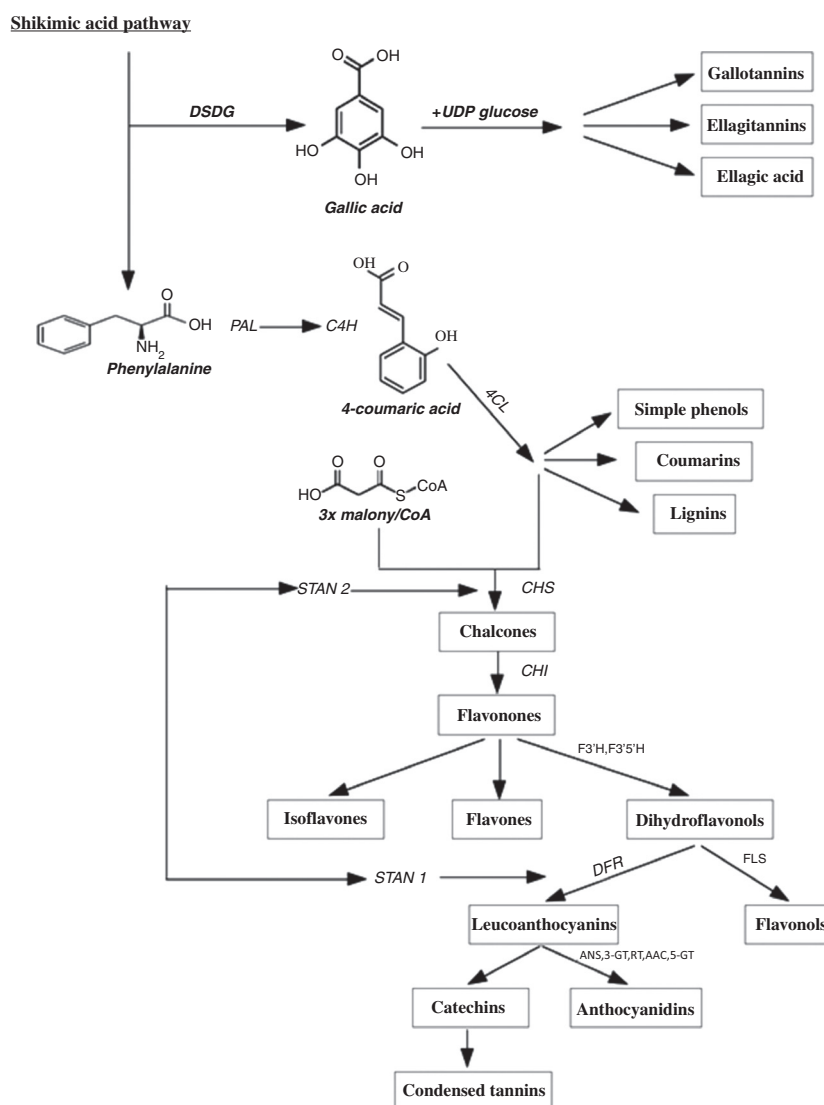


Figure 1. Biosynthesis of phenolic compounds (modified after Stushnoff et al. 2010; Al-Sane et al. 2011; Bai et al. 2011; Lachman et al. 2012). ANS, anthocyanidin synthase; CHI, chalconeisomerase; CHS, chalconesynthetase; C4H, cinnamic acid 4-hydroxylase; DFR, dihydroflavonol-4-reductase; DSDG, dehydroshikimate dehydrogenase; 4CL, 4-coumarate-CoA ligase; PAL, phenylalanine ammonia lyase; STAN, *Solanum tuberosum* anthocyaninsynthase; UDP glucose, 3-O-flavonoid glucosyltransferase.

biosynthesis was performed using selected markers for *ans*, *stan1*, and *chi* genes.

This work is focused on comparing the chemical composition with botanical classification based on morphological characteristic and the similarities between the involved genes. Our hypothesis was that there is an association between the similarities, the involved genes, and the concentration patterns of anthocyanidins and antioxidants.

Material and Methods

Collection of potatoes

Thirteen purple potatoes belonging to three species (*Solanum curtilobum*, *S. ajanhuiri*, and *S. tuberosum* subsp. *andigenum*) (Fig. 2) were harvested in April 2011 during one day at Quipaquipani Research and Training Center (PROINPA Foundation). The station is located 4 km south of the town of Viacha (coordinates 68°17'49", 16°40'17"), Ingavi Province in the Department of La Paz 3870 m.a.s.l. Each sample was taken from separate clones grown at a section of a field. The sample was obtained through a random collection of potatoes from the growing section until 1 kg was obtained. The samples were stored in plastic bags at 5°C for 6 h and then transferred to the laboratory.

Extraction (TAC and TPH)

The potatoes were quickly washed and dried, then cut into small cubes, and about 3 g was weighed and extracted with 27 mL of methanol:water (9:1, by volume) by vortexing followed by sonication of the sample in an ice-water bath (0°C, 15 min). The mixture was centrifuged in a Thermo IEC Multi/RF with an 8850 rotor (Thermo Fisher Scientific Inc., Waltham, MA) at 20,000g for 30 min at 4°C, and the aspirated supernatant was stored at −80°C. The supernatants were evaluated by the ABTS (2,2'-azino-bis[3-ethylbenzothiazoline-6-sulfonic acid]) and TPH methods.

Extraction (HPLC)

The potatoes were quickly washed and dried, then cut into small cubes, and about 50 g was weighed, frozen at −20°C, and lyophilized for 3 days. The dry structure was grounded and kept ready for ethanol/water extraction. One gram of each lyophilized sample was extracted with 10 mL of methanol:water (9:1, by volume) by vortexing, followed by sonicating the sample in an ice-water bath (0°C, 15 min). The mixture was centrifuged at 20,000g for 30 min at 4°C, and the aspirated supernatant was stored at −80°C. The supernatants were hydrolyzed and evaluated by HPLC.



Figure 2. Colored potatoes collected in Bolivian Altiplano. Samples 1, 2, and 3 belong to “Ñojchajachaya” cultivar, samples from 4 to 10 belong to “Caty” cultivar, 11 belongs to “Chocapito-morado” cultivar, 12 belongs to “Ajanwiri (clon)” cultivar, and 13 belongs to “Durazno Imilla 30 CN-010” cultivar.

TAC evaluated by the ABTS and TPH methods

Folin-Ciocalteu reagent, gallic acid, sodium carbonate, and acetone were purchased from Merck (Darmstadt, Germany). ABTS, potassium persulfate, Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid, 97%), and TPTZ (2,4,6-tripyridyl-s-triazine) were obtained from Sigma-Aldrich (St. Louis, MO), ferric chloride from ICN Biomedicals (Costa Mesa, CA), glacial acetic acid and sodium acetate from BDH Chemicals (Poole, U.K.) and methanol from Laboratory Supplies (Poole, U.K.). All chemicals were of analytical grade. To oxidize the colorless ABTS to the blue-green ABTS⁺ radical cation, 5 mL of ABTS solution (7 mmol/L) was mixed with 88 μ L of K₂S₂O₈ (140 mmol/L) and stored at room temperature in the dark overnight. On the day of the analysis, the ABTS⁺ radical cation solution was diluted with acetate buffer to reach an absorbance of 0.70 (\pm 0.02) at 734 nm. A Trolox standard stock solution, 5 mmol/L in ethanol, was diluted with acetate buffer to concentrations of 20–200 μ mol/L. The extraction follows Nilsson *et al.* (2005) using modifications suggested by Peñarrieta *et al.* (2008). Different standards or samples (100 μ L) were added to 1 mL of ABTS⁺ solution, mixed for 30 sec, after which the absorbance reading was started after another 30 sec and maintained for 6 min at 734 nm and 25°C. The concentration was plotted against percent inhibition, which was used for the calculation. The results are expressed as μ mol Trolox equivalents per gram of dry matter (μ mol TE/g dm).

Total phenolic compounds were measured by the Folin-Ciocalteu reagent which oxidizes the phenolic compounds to phenolates at alkaline pH in a saturated solution of sodium carbonate, resulting in a blue molybdenum–tungsten complex (Singleton and Rossi 1965). However, the method has a limited specificity and a number of substances (particularly ascorbic acid and fructose) (Prior *et al.* 2005) can be assumed to contribute to the apparent result (Lachman *et al.* 2008a). Lachman *et al.* (2008) analyzed the interference systematically and provided data of reaction absorbances of potentially interfering substances. Using these data and data on fructose (Mano *et al.* 2007), the interference can be estimated to be less than 5%.

The Folin-Ciocalteu reagent was diluted with water (1:10 by volume) prior to analysis. A gallic acid stock solution was prepared in 80% aqueous acetone (1:1 by volume), and the gallic acid standard curve was diluted with water to concentrations of 235–1180 μ mol/L. From each standard solution and sample, 50 μ L was mixed with 2.5 mL of Folin-Ciocalteu reagent and 2.0 mL of sodium carbonate solution. The samples were mixed and incubated at 45°C for 30 min. The absorbance was read at 765 nm after cooling at room temperature. The

absorbance of each sample was compared with the values obtained from a calibration curve using gallic acid as standard. The results are expressed as μ mol gallic acid equivalents per gram of dry matter (μ mol GAE/g dm) (Peñarrieta *et al.* 2008, 2009, 2011).

HPLC

Baicalein (98%) internal standard, ferulic acid (99%), caffeic acid (98%), chlorogenic acid (95%), gallic acid (99%), and syringaldehyde (99%) were obtained from Sigma-Aldrich. Cyanidin and pelargonidin were obtained from Extrasynthese (Genay, France), and hydrochloric acid (36.5–38.0%) was obtained from J.T. Baker (México city, México).

Before analyzing the samples in HPLC, they were hydrolyzed by refluxing 250 μ L methanol:water (9:1) extracts with 250 μ L 3 N HCl and 250 μ L of baicalein (internal standard) for 1 h at 90 C.

Most of the polyphenolic compounds are bound to insoluble polysaccharide components and the hydrolysis is used to release polyphenols from their corresponding glycosides.

The chemical instability during extraction and analyses of anthocyanidins is a particular concern (Reyes *et al.* 2004). To protect the components the samples were stored frozen, lyophilized, and the HPLC was run directly after the hydrolysis (the acid condition also stabilizes the anthocyanidins) (Fuhrman 2001; Reyes *et al.* 2004). The HPLC analysis was made in methanol–water extracts from lyophilized samples.

The polyphenolic compounds were separated using an Agilent liquid chromatographic system comprising a vacuum degasser (G1322 A), a solvent delivery system (Quat Pump-G1311A), an auto-injector ALS (ALS-G1329A), a column oven (ColCom-G1316A), and a diode array detector (G1315B) (Agilent, Santa Clara, CA). The column was a reverse-phase Agilent eclipse plus C18 (150 \times 4.6 mm) protected by a 10 mm precolumn. The flow rate was 0.8 mL/min and the injection volume was 20 μ L. The mobile phase was a binary solvent system consisting of (A) 1% acetic acid/water and (B) methanol and the gradient used was 40% B at 0 min, 65% B after 5 min, 90% B after 10 min, and 40% B after 15 min until 17 min. The UV absorbance of the eluate was recorded using a multiple diode array detector (190–550 nm). Retention times and absorbance spectrum profiles were compared with standards. Pure standards were also added to the samples as control and peak splitting was used to confirm the identification (Carrasco *et al.* 2011; Peñarrieta *et al.* 2011). The samples were quantified at 310 and 530 nm using baicalein as internal standard and using pure standards for specific absorbance determination (Peñarrieta *et al.* 2008).

DNA extraction

Tris acetate, tris base, EDTA, agarose, blue dye, green dye, ethidium bromide, Flexi taq polymerase, and Mix dNTPs were obtained from Promega (Madison, WI). Sodium acetate was obtained from Sigma-Aldrich GmbH (Steinheim, Germany) and isopropanol was from Merck AG (Darmstadt, Germany).

The DNeasy Plant mini kit was obtained from Qiagen GmbH (Hilden, Germany), BigDye Terminator v3.1 Cycle Sequencing kit was obtained from AB Applied Biosystem (Foster City, CA), 100-bp DNA ladder was obtained from Solis BioDyne (Tartu, Estonia), and the markers *21ab*, *ans*, *bch6*, *chi*, *chs*, *ct203*, *gp24*, *stan1*, *ugpase*, and *zep* were obtained from Eurogentec (San Diego, CA).

DNA was isolated from 50 mg of lyophilized sample of purple potatoes. Around 50 g of fresh sample was frozen at -20°C and had been lyophilized by LabconcoFreeZone 4.5 that reach -50°C (Labconco, Kansas, MO) and grounded into powder in mortar. A DNeasy Plant Mini extraction kit (Qiagen GmbH) was then used for DNA isolation (according to the manufacturer's instructions). The DNA quality was verified by electrophoresis on 1% agarose gel and quantified using a spectrophotometer.

Polymerase chain reaction

Polymerase chain reaction (PCR) amplification was made using 40 ng of DNA and Master Mix (Promega), with the following concentrations: 0.2 mmol/L dNTPs, 1.5 mmol/L MgCl_2 , 0.03 $\mu\text{L}/\mu\text{L}$ Taq DNA polymerase (Promega), and 1 $\mu\text{mol}/\text{L}$ for primers (forward and reverse), respectively. Thermal cycling conditions were found by BIOER thermocycler for 10 primers referred (Zhang *et al.* 2009) those which are on a range of 400–900 bp: *bch6*, *21ba*, *ugp-ase*, *ans*, *ct-203*, *chi*, and *gp-24* and the following conditions: 3 min denaturation at 95°C , 1 min annealing in the range of 48 – 57°C and 1 min, 30 sec extension at 72°C , and the number of cycles was between 30 and 33. For primers in the range 1000–1600 bp: *stan1*, *chs*, and *zep*, the conditions were 2 min denaturation at 95°C , 1 min annealing in the range 62 – 65°C and 1 min, 30 sec extension at 72°C , and 35 cycles. The products obtained after PCR optimization were evaluated by horizontal electrophoresis in agarose gel (2%), stained with ethidium bromide (0.1 $\mu\text{L}/\text{mL}$; Promega), and visualized with a ultraviolet light transilluminator with the addition of a molecular weight marker 100 to 3000 bp (100-bp DNA ladder; Solis Biodyne).

Sequence analysis

Phylogenetic trees were made based on *ans* and *stan1* genes. Primers reported by Zhang *et al.* (2009) were used

to amplify partial regions of *ans* and *stan1*. Genes and the amplicons were purified using Qiagen DNA Cleanup Systems and sequenced. Raw sequences were imported in Geneious Pro (Ver. 5.4.6, Biomatters Ltd., Auckland, New Zealand) and homology searches were done by the Basic Local Alignment Search Tool (BLAST) to identify protein-coding regions (exons). Exon sequences were imported in MEGA 5 and aligned using multiple sequence alignment – CLUSTALW (Tamura *et al.* 2011). The phylogenetic trees were built with the maximum composite likelihood model and the neighbor-joining method (Tamura *et al.* 2011). Bootstrap analysis was conducted with 1000 replicates. The *Ans* phylogenetic tree was constructed based on the following genes: *ans10*, *ans12*, *ans7*, *ans6*, *ans4*, *ans5* (GenBank accession number: KC752436), *ans9*, and *ans8*. It was divided in three clades called B1, B2, and B3 originating from a common ancestor, the same for *stan1* phylogenetic tree, respectively. The *stan* phylogenetic tree was constructed based on the following genes: *stan12*, *stan9*, *stan3*, *stan1*, *stan2*, *stan10* (GeneBank accession number: KC752437), *stan6*, *stan8*, *stan5*, and *stan11*. It was divided in three clades called A1, A2, and A3 originating from a common ancestor.

Statistical analysis

The principal component analysis (PCA) was performed using MATLAB Release 2012a (MathWorks, Natick, MA).

Results and Discussion

Chemical composition of different cultivars

TAC and TPH of colored potatoes are shown in Figure 3, ranging from 1.0 to 7.0 μmol Trolox/g dry weight (dw) and 8.0–17.0 μmol GAE/g dw, respectively. The TAC values obtained are similar to those reported in the literature for potato (1 μmol Trolox/g dw) (Peñarrieta *et al.* 2011) as shown in Table 1. The TPH results are similar to those reported previously for potatoes (10 μmol GAE/g dw) (Peñarrieta *et al.* 2011) as well as of purple-fleshed potatoes (19.3 μmol GAE/g dw) (Lachman *et al.* 2008a,b) in Stachy locality of Czech Republic with environmental conditions similar to that in the Andean region (lower temperature and higher altitude; see Table 1).

The polyphenols were identified and quantified (Table 2) by comparison with standards using HPLC with a UV–Vis diode array detector. Seven individual compounds were identified: cyanidin, pelargonidin, ferulic acid, caffeic acid, chlorogenic acid, gallic acid, and syringaldehyde (Figure 4). Baicalein was used as the internal standard (Peñarrieta *et al.* 2011).

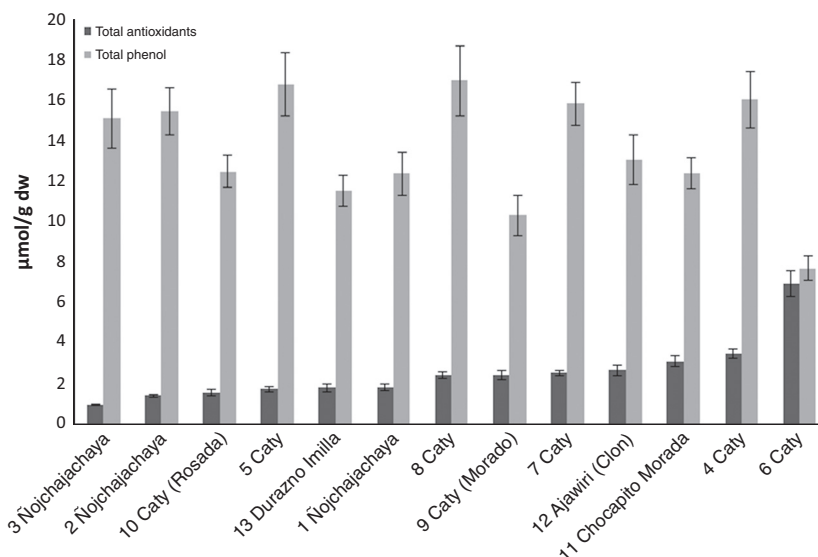


Figure 3. Total antioxidants expressed as trolox equivalent/g dry weight and total phenols expressed as gallic acid equivalent/g dry weight of different colored potato. Values are means of triplicate measurements \pm standard error of mean.

Table 1. Comparison of present study data in colored potato with literature data.

<i>Solanum</i> cultivar	TAC (μmol Trolox/g dw)	TPH (μmol GAE/g dw)
This study		
<i>S. stenotomum</i> (colored potato)	1–7	8–17
Literature data		
<i>S. tuberosum</i> (colored potato) ¹		9–35
<i>S. tuberosum</i> L. (purple-flesh potato) ²		19
<i>S. tuberosum</i> L. ³	1	10

¹TPH evaluated by Folin-Ciocalteu reagent (Andre et al. 2007).

²TPH evaluated by Folin-Ciocalteu reagent made by ABTS method (Lachman et al. 2008, 2008).

³TAC evaluated by ABTS method and TPH by Folin-Ciocalteu reagent (Peñarrieta et al. 2011).

The absorption maxima for cyanidin and pelargonidin were found at 530 nm and for ferulic acid, caffeic acid, chlorogenic acid, gallic acid, and syringaldehyde at 310 nm. The presence of pelargonidin and cyaniding has been reported in Shetland Black cultivar purple-flesh potato by Lachman et al. (2012). Also, the polyphenols identified in this study have been reported before in purple-fleshed potatoes (Friedman 1997; Lewis et al. 1998; Del Mar Verde Méndez et al. 2004).

The quantitative data are summarized in Table 2. The amount of individual polyphenolic compound varies between cultivars. Gallic acid is the main polyphenolic compound in all samples followed by chlorogenic acid

and caffeic acid, while ferulic acid is present at lower concentrations. The caffeic acid concentration is similar among the samples, while cyanidin and pelargonidin levels are higher in some of the “Nojchajachaya” samples (Table 2).

Pelargonidin values obtained (0.004–0.07 $\mu\text{mol/g}$ dw) were comparable to that found in the Shetland Black cultivar purple-flesh potato (0.02 $\mu\text{mol/g}$ dw) (Lachman et al. 2012), but the results were lower than those reported in the literature for red potato (2.3–23.3 $\mu\text{mol/g}$ dw) (Lewis et al. 1998). Also, cyanidin values (0.003–0.07 $\mu\text{mol/g}$ dw) were comparable to that found in Shetland Black cultivar (0.01 $\mu\text{mol/g}$ dw), which was the only purple-flesh potato with both anthocyanidins reported (Lachman et al. 2012).

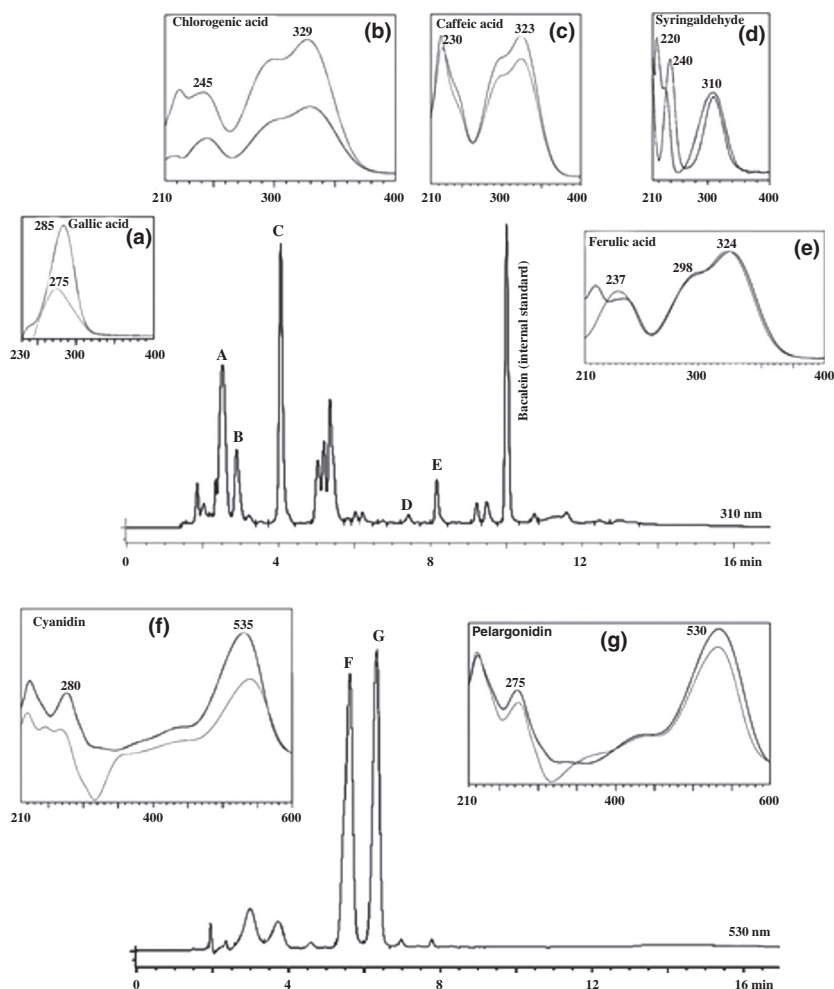
The ferulic acid content (0.01–0.04 $\mu\text{mol/g}$ dw) is in agreement with literature values for pink potato (0.01 $\mu\text{mol/g}$ dw) (Del Mar Verde Méndez et al. 2004). The caffeic acid values (0.03–0.1 $\mu\text{mol/g}$ dw) are also in agreement with literature data obtained from pink potato (0.1 $\mu\text{mol/g}$ dw) (Del Mar Verde Méndez et al. 2004). On the other hand, lower values were obtained for chlorogenic acid content in this study (0.04–0.5 $\mu\text{mol/g}$ dw) compared with literature data (1 $\mu\text{mol/g}$ dw) (Del Mar Verde Méndez et al. 2004; Peñarrieta et al. 2011).

The values for gallic acid (1.2–7.6 $\mu\text{mol/g}$ dw) are slightly higher than previously reported data (1.1 $\mu\text{mol/g}$ dw). The values for syringaldehyde (0.01 $\mu\text{mol/g}$ dw) are similar to values reported previously (Peñarrieta et al. 2011).

PCA was used to reveal potential patterns between the 13 colored potato samples and TAC, TPH, concentrations

Table 2. Content of individual polyphenolic compounds in colored potato samples in methanol–water extracts, expressed as $\mu\text{mol/g dw}$.

Cultivar	Sample	Cyanidin	Pelargonidin	Ferrulic acid	Caffeic acid	Chlorogenic acid	Gallic acid	Syringaldehyde
Ñojchajachaya	1	0.07	0.07	0.01	0.09	0.3	1.5	0.01
Ñojchajachaya	2	0.04	0.06	0.01	0.08	0.2	1.6	0.00
Ñojchajachaya	3	0.01	0.01	0.02	0.07	0.3	4.1	0.00
Caty	4	0.01	0.005	0.02	0.08	0.5	1.5	0.00
Caty	5	0.02	0.02	0.04	0.13	0.4	7.6	0.00
Caty	6	0.003	0.03	0.01	0.06	0.2	1.2	0.00
Caty	7	0.02	0.01	0.02	0.10	0.5	3.4	0.00
Caty	8	0.01	0.03	0.02	0.07	0.2	1.8	0.00
Caty (Morado)	9	0.02	0.02	0.03	0.09	0.2	5.1	0.00
Caty (Rosada)	10	0.03	0.01	0.01	0.07	0.3	2.6	0.00
Chocapito-Morada	11	0.05	0.02	0.01	0.08	0.2	2.9	0.00
Ajawiri (Clon)	12	0.03	0.01	0.01	0.06	0.2	2.9	0.00
Durazno Imilla	13	0.01	0.004	0.01	0.03	0.04	3.7	0.00
Median		0.02	0.02	0.01	0.08	0.2	2.9	0.00
SD		0.02	0.02	0.01	0.02	0.1	1.8	0.00
SEM		0.01	0.01	0.002	0.01	0.04	0.5	0.001
Range		<0.003–0.07	<0.004–0.07	<0.01–0.04	<0.03–0.13	<0.04–0.5	<1.2–7.6	<0.0–0.01

**Figure 4.** Chromatogram of polyphenolic compounds identified in colored potatoes. Black peak spectra belong to the standards and gray peaks belong to the samples.

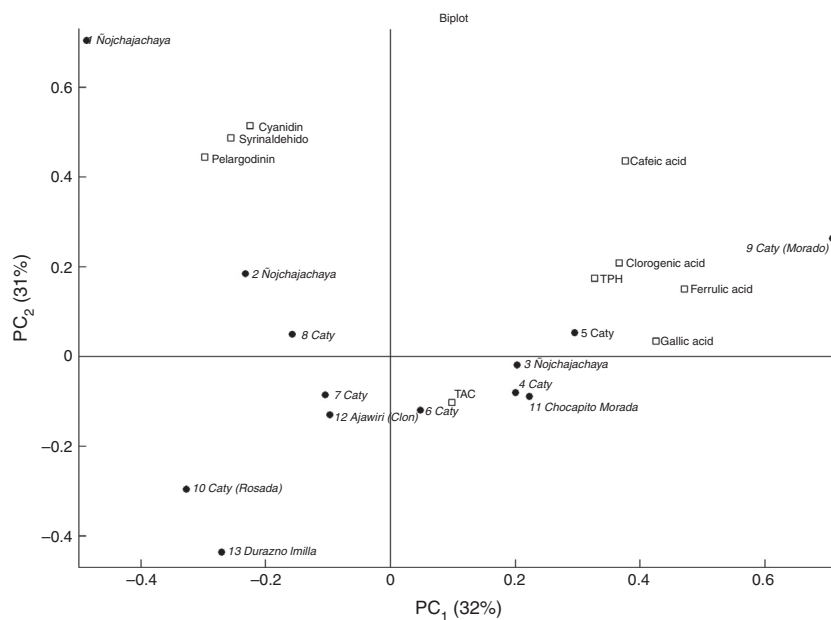


Figure 5. PCA analysis (biplot) of anthocyanin, phenolic compounds, total phenol, and total antioxidant content in 13 different purple potatoes.

of individual anthocyanidins and polyphenolic compounds are the nine measured parameters. The explained total variance of PC1 is 32% and of PC2 is 32% (Fig. 5).

The “Nojchajachaya” group is scattered along the second PC, with some samples displaying a high concentration of cyanidin, pelargonidin, and syringaldehyde, whereas one sample displays low concentrations. These samples also have similar levels of TAC (Fig. 3). The Caty group is scattered around the center of the coordinates and therefore does not display any pattern. The results show that the botanical classification only describes limited aspects of the variability of this material.

Analysis of genes associated with the biosynthesis of anthocyanidin

In the beginning about 10 genes associated with anthocyanidin were analyzed (Fig. 6). However, only three (*chi*, *ans*, and *stan1*) of the 10 genes are directly associated with the biosynthesis of anthocyanidin in colored potato. The three genes, *chi*, *ans*, and *stan1*, were present in all individual samples as confirmed by gel electrophoresis (Fig. 6). The three genes of each sample were sequenced. The size of the fragments (500 bp) of the *chi* gene material was too small to allow for a proper phylogenetic analysis. The phylogenetic trees were made for *ans* (800 bp) and *stan1* (1200 bp) sequences. For some samples, individual samples had to be excluded due to poor resolution; for the *ans* gene, only eight could be used and for the *stan1* gene only 10 could be used of the 13 individual samples.



Figure 6. Genomic DNA of Durazno Imilla 30CN-010 sample was amplified by *Bch6*, *21BA*, *UGPasa*, *CT-203*, *Chi*, *GP-24*, *Stan1*, *Chs*, and *Zep* primers, restricted with Taq DNA polymerase, and electrophoresed through a 2% agarose gel. The primers are listed in ascending order in a range of 400–1600 bp.

The *stan1*, from here on only termed *stan*, phylogenetic tree clearly shows how samples of the *stan* gene are grouped together, how they originate from the same node, and that they are completely separated from *Nicotiana tabacum*, thereby confirming the uniformity in the material making contamination unlikely (Fig. 7).

The *stan* phylogenetic tree is divided into three clades by common nodes. The genes *stan12* (i.e., the *stan* gene from sample 12), *stan9*, *stan3*, *stan1*, *stan2*, and *stan10* are

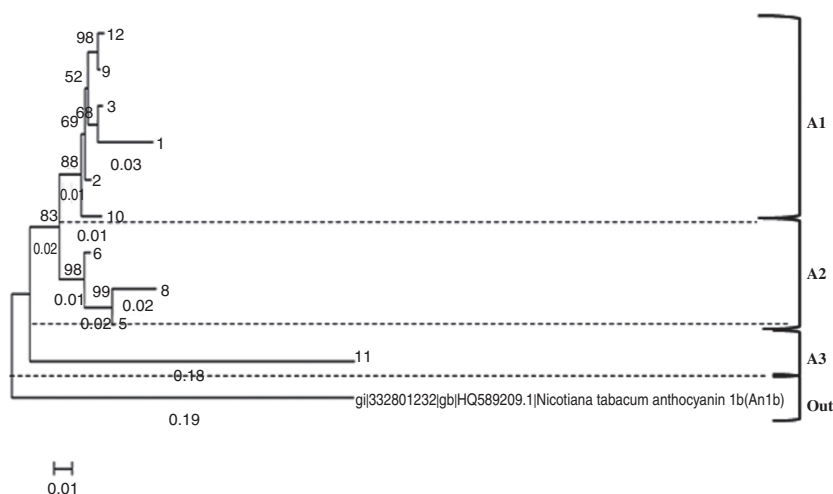


Figure 7. *Stan* gene phylogenetic tree constructed by coding exon partial area with approximately 300–500 base pairs made by the Neighbor-Joining algorithm.

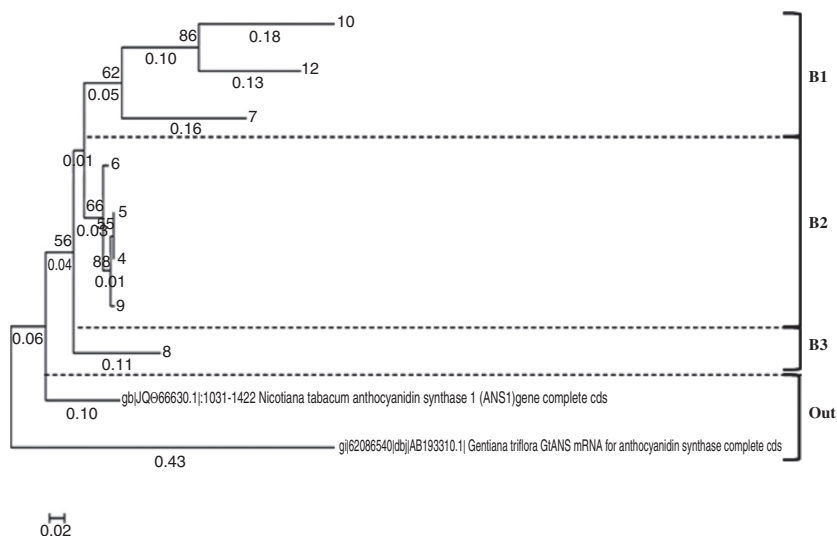


Figure 8. *Ans* gene phylogenetic tree constructed by coding exon partial area with approximately 300–500 base pairs made by the Neighbor-Joining algorithm.

grouped in the same clade called A1 and they have 88% similarity. In clade A2 the genes *stan6*, *stan8*, and *stan5* are branched together with a common node with a similarity above 98%. Clade A3 (*stan11*) shows 83% similarity with clades A1 and A2.

The *ans* phylogenetic tree can be divided into three clades (B1, B2, and B3) that are grouped together and are well separated from *N. tabacum* and *Gentiana triflora* (Fig. 8). In clade B1 there are genes from three samples *ans10*, *ans12*, and *ans7*, which belong to a common node with a 62% of similarity. Clade B2 is formed by *ans6*, *ans4*, *ans5*, and *ans9*, with a common node, and clade B3 is formed by *ans8* with 55% similarity to clades B1 and B2.

The explanatory power of the analysis of the *ans* genes of samples may reflect the larger biological variation in the *ans* gene compared to the *stan* gene.

The botanical classification is based on morphological characteristics of the tubers (Table 3), and the *stan* and *ans* phylogenetic trees (Figs. 7, 8) show no direct similarity as the different cultivars are not grouped in the phylogenetic trees.

Correlation between phylogenetic trees and chemical composition

PCA was used to reveal patterns among clades from *stan* and *ans* phylogenetic trees and the chemical composition

Table 3. Description of potatoes (*Solanum* sp.) collected in La Paz, Bolivia.

Code	Cultivar	Scientific name	Dry matter (%)
1	Ñojchajachaya	<i>S. tuberosum</i> subsp. <i>andigenum</i>	33
2	Ñojchajachaya	<i>S. tuberosum</i> subsp. <i>andigenum</i>	26
3	Ñojchajachaya	<i>S. tuberosum</i> subsp. <i>andigenum</i>	29
4	Caty	<i>S. tuberosum</i> subsp. <i>andigenum</i>	31
5	Caty	<i>S. tuberosum</i> subsp. <i>andigenum</i>	28
6	Caty	<i>S. tuberosum</i> subsp. <i>andigenum</i>	24
7	Caty	<i>S. tuberosum</i> subsp. <i>andigenum</i>	27
8	Caty	<i>S. tuberosum</i> subsp. <i>andigenum</i>	33
9	Caty (Morado)	<i>S. tuberosum</i> subsp. <i>andigenum</i>	28
10	Caty (Rosada)	<i>S. tuberosum</i> subsp. <i>andigenum</i>	31
11	Chocapito -Morada	<i>S. curtilobum</i>	25
12	Ajawiri (Clon)	<i>S. ajanhuiri</i>	26
13	Durazno Imilla 30CN-010	<i>S. tuberosum</i> subsp. <i>andigenum</i>	32

of individual samples. The PCA of the eight *stan* samples gave PC1 and PC2 together explaining 64% of the total variance (Fig. 9). The three clades A1, A2, and A3 enclose each other. Thus, this result shows that the variance in the chemical composition is not explained by differences in the *stan* gene.

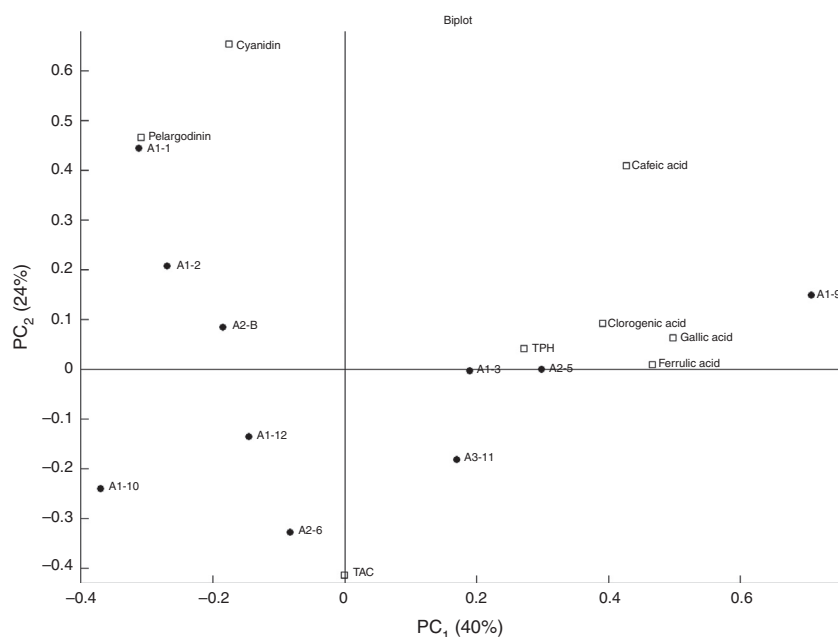
Figure 10 shows the corresponding PCA for the *ans* gene for the eight samples where PC1 and PC2 together explain 71% of the variance. Clades B1 and B2 are from two separated areas, indicating that the *ans* gene has a

clear impact on the chemical composition. Clade B1 has a correlation with a somewhat higher level of pelargonidin. B2 is located along the positive PC1 and thereby correlates with high levels of ferulic acid, gallic acid, caffeic acid, chlorogenic acid, and TPH (Fig. 10).

The *ans* gene is directly involved in anthocyanidin synthesis. The anthocyanidin has a more final position in the biochemical pathways (Singleton and Rossi 1965; Nilsson et al. 2005; Mano et al. 2007; Peñarrieta et al. 2008; Davies et al. 2009).

The *stan* gene controls dihydroxyflavonol-4-reductase activity (Bai et al. 2011; Lachman et al. 2012) that leads to the formation of leucoanthocyanins that may be converted into anthocyanidins or into catechins and condensed lignins (Fig. 1). Thus a less clear connection can be assumed. Thus potato color is confirmed as a sign not only of high level of anthocyanidins but also of polyphenols in general (Fuhrman 2001).

Naturally, not only the genetics are expected to determine the chemical composition as other aspects – *extrinsic factors*: environmental conditions, cooler temperatures (Reyes et al. 2004) growing conditions (Lachman et al. 2008a; Hamouz et al. 2010) – and the *intrinsic factor*: the cultivar (Andre et al. 2007; Hamouz et al. 2010) – may play a role. However, the samples in this article were collected at an experimental farm with uniform growing conditions, which should limit the influence of extrinsic factors on the results.

**Figure 9.** PCA analysis (biplot) of chemical composition in 10 different purple potatoes and clades belonging to the *stan* phylogenetic tree.

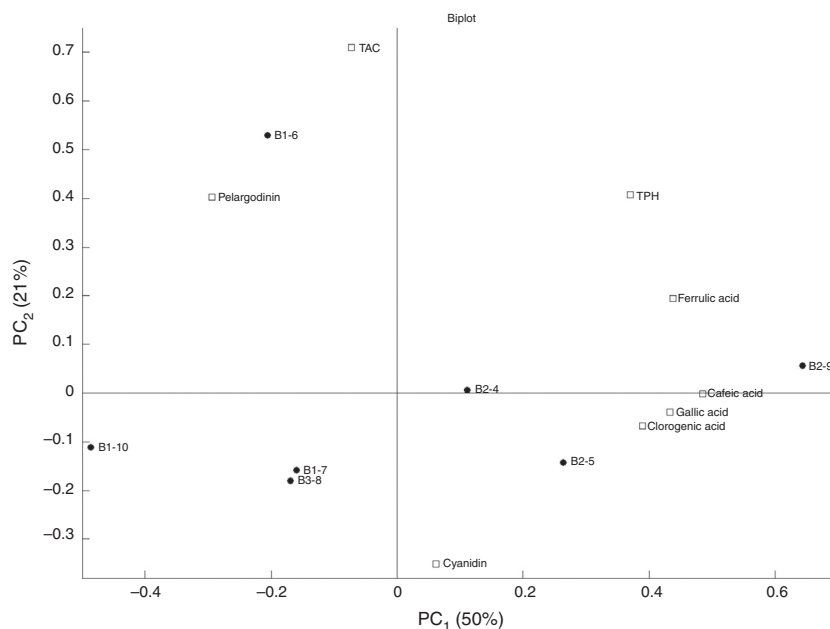


Figure 10. PCA analysis (biplot) of chemical composition in seven different purple potatoes and clades belonging to the *ans* phylogenetic tree.

Conclusion

The results of this study show the existence of a limited correlation between botanical classification, based on morphological identification, and polyphenol composition. No correlation between genetic grouping of the *ans* and *stan* genes and botanical classification was found. However, it was possible to identify a correlation between the *ans* gene clades and the levels of anthocyanidins as well as of other polyphenols. Thus, this result confirms the concept that potato color can be used in the search for high polyphenol potato cultivars.

Acknowledgments

The study was supported by the Swedish International Development Agency (SIDA) in a collaborative project between San Andrés University (Bolivia) and Lund University (Sweden). We thank Alejandro Bonifacio, researcher from Quipaquipani Research and Training Center, PROINPA Foundation, for cooperation and for providing the samples. We also thank Javier Linares, Ph.D., researcher from Biotechnology Department, Faculty of Engineering (LTH), for editing the sequences by Chromas Pro Version 1.5 program.

Conflict of Interest

None declared.

References

- Alcalde-Eon, C., G. Saavedra, S. Pascual-Teresa, and J. Rivas-Gonzalo. 2004. Identification of anthocyanins of pinta boca (*Solanum stenotomum*) tubers. *Food Chem.* 86:441–448.
- Al-Sane, K. O., G. Povero, and P. Perata. 2011. Anthocyanin tomato mutants: overview and characterization of an anthocyanin-less somaclonal mutant. *Plant Biosyst.* 145: 436–444.
- Andre, C., M. Ghislain, P. Bertin, M. Oufir, M. R. Herrera L. Hoffman, et al. 2007. Andean potato cultivars (*Solanum tuberosum* L.) as a source of antioxidant and mineral micronutrients. *J. Agric. Food Chem.* 55:366–378.
- Bai, Y., S. Pattanaik, B. Patra, J. Werkman, C. Xie, and L. Yuan. 2011. Flavonoid-related basic helix-loop-helix regulators, NtAn1a and NtAn1b, of tobacco have originated from two ancestors and are functionally active. *Planta* 234:363–375.
- Brown, C. 2005. Antioxidant in potato. *Am. J. Potato Res.* 82:163–172.
- Carrasco, C., H. Baudel, M. Peñarrieta, C. Solano, L. Tejada, C. Roslander, et al. 2011. Steam pretreatment and fermentation of the straw material “Paja Brava” using simultaneous saccharification and co-fermentation. *J. Biosci. Bioeng.* 111:167–174.
- Davies, K. M., H. Zhang, and K. E. Schwinn. 2009. Recent advances in polyphenol research. Pp. 139–166 in F. Daayf and V. Lattanzio, eds. *Recent advances in the molecular*

- biology and metabolic engineering of flavonoid biosynthesis in ornamental plants [online]. Wiley-Blackwell, Oxford, U.K.
- Del Mar Verde Méndez, C., M. Á. Rodríguez Delgado, E. M. Rodríguez Rodríguez, C. Díaz Romero. 2004. Content of free phenolic compounds in cultivars of potatoes harvested in Tenerife (Canary Islands). *J. Agric. Food Chem.* 52:1323–1327.
- Deroles, S. 2009. Anthocyanin biosynthesis in plant cell cultures: a potential source of natural colorants. Pp. 108–167 in C. Winefield, K. Davies, and K. Gould, eds. *Anthocyanins: biosynthesis, functions, and applications*. Springer, Palmerston North, New Zealand.
- Friedman, M. 1997. Chemistry, biochemistry, and dietary role of potato polyphenols. *Agric. Food Chem.* 45: 1523–1540.
- Fuhrman, B., and M. Aviram. 2001. Polyphenols and flavonoids protect LDL against atherogenic modifications. Pp. 303–336 in E. Cadenas and L. Packer, eds. *Handbook of antioxidants*. Rambam Medical Center, Haifa, Israel.
- Hamouz, K., J. Lachman, A. Hejtmankova, K. Pazderu, M. Cizek, and P. Dvorak. 2010. Effect of natural and growing conditions on the content of phenolics in potatoes with different flesh colour. *Plant Soil Environ.* 56:368–374.
- Hamouz, K., J. Lachman, K. Pazderu, J. Tomasek, K. Hejtmankova, and V. Pivec. 2011. Differences in anthocyanin content and antioxidant activity of potato tubers with different flesh colour. *Plant Soil Environ.* 57:478–485.
- Lachman, J., M. Šulc, M. Orsák, K. Hamouz, and P. Dvořák. 2008a. Differences in phenolic content and antioxidant activity in yellow and purple-fleshed potatoes grown in the Czech Republic. *Plant Soil Environ.* 54:1–6.
- Lachman, J., K. Hamouz, M. Orsák, V. Pivec, and P. Dvorak. 2008b. The influence of flesh colour and growing locality on polyphenolic content and antioxidant activity in potatoes. *Sci. Hort.* Amsterdam 117:109–114.
- Lachman, J., K. Hamouz, M. Orsák, V. Pivec, K. Hejtmankova, K. Pazderu, et al. 2012. Impact of selected factors – cultivar, storage, cooking and baking on the content of anthocyanins in coloured-flesh potatoes. *Food Chem.* 133:1107–1116.
- Lewis, C., J. Walker, J. Lancaster, and K. Sutton. 1998. Determination of anthocyanins, flavonoids and phenolic acids in potatoes. I: Colored cultivars of *Solanum tuberosum* L. *Sci. Food Agric.* 77:45–57.
- Mano, H., F. Ogasawara, K. Sato, H. Higo, and Y. Minobe. 2007. Isolation of regulatory gene of anthocyanin biosynthesis in tuberous roots of purple-fleshed sweet potato. *Plant Physiol.* 143:1252–1268.
- Nilsson, J., D. Pillai, G. Önning, C. Persson, Å. Nilsson, and B. Åkesson. 2005. Comparison of the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and ferric reducing anti-oxidant power (FRAP) methods to assess the total antioxidant capacity in extracts of fruit and vegetables. *Mol. Nutr. Food Res.* 49:239–246.
- Peñarrieta, J., J. Alvarado, B. Åkesson, and B. Bergenståhl. 2008. Total antioxidant capacity and content of flavonoids and other phenolic compounds in canihua (*Chenopodium pallidicaule*): an Andean pseudocereal. *Mol. Nutr. Food Res.* 52:708–717.
- Peñarrieta, J., J. Alvarado, B. Bergenståhl, and B. Åkesson. 2009. Total antioxidant capacity and content of phenolic compounds in wild strawberries (*Fragaria vesca*) collected in Bolivia. *Int. J. Fruit Sci.* 9:344–359.
- Peñarrieta, J., T. Salluca, L. Tejeda, J. Alvarado, and B. Bergenståhl. 2011. Changes in phenolic antioxidants during chuño production (traditional Andean freeze and sun-dried potato). *J. Food Compos. Anal.* 24:580–587.
- Preedy, V. R., R. R. Watson, and V. Patel. 2011. Nuts and seeds in health and disease prevention. Elsevier Academic Press Inc., San Diego, CA.
- Prior, R., X. Wu, and K. Schaich. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.* 53:4290–4302.
- Reyes, L. F., J. C. Miller Jr., and L. Cisneros-Zevallos. 2004. Environmental conditions influence the content and yield of anthocyanins and total phenolics in purple- and red-flesh potatoes during tuber development. *Am. J. Potato Res.* 81:187–193.
- Singleton, V., and J. Rossi. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagent. *Am. J. Enol. Vitic.* 16:144–158.
- Stushnoff, C., L. J. M. Ducreux, R. D. Hancock, P. E. Hedley, D. G. Holm, G. J. McDougall, et al. 2010. Flavonoid profiling and transcriptome analysis reveals new gene–metabolite correlations in tubers of *Solanum tuberosum* L. *J. Exp. Bot.* 61:1225–1238.
- Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kamur. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28:2731–2739.
- Zhang, Y., C. Jung, and W. De Jong. 2009. Genetic analysis of pigmented tuber flesh in potato. *Theor. Appl. Genet.* 119:143–150.