Food Chemistry 229 (2017) 837–846

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Chlorogenic acid, anthocyanin and flavan-3-ol biosynthesis in flesh and skin of Andean potato tubers (*Solanum tuberosum* subsp. *andigena*)

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ARTICLE INFO

Article history:
Received 26 August 2016
Received in revised form 22 February 2017
Accepted 28 February 2017
Available online 2 March 2017

Keywords:
Anthocyanidin reductase
Leucoanthocyanidin reductase
Antioxidant
Phenolic compounds metabolism
Solanum tuberosum

ABSTRACT

Natural variation of Andean potato was used to study the biosynthesis of phenolic compounds. Levels of phenolic compounds and corresponding structural gene transcripts were examined in flesh and skin of tubers. Phenolic acids, mainly chlorogenic acid (CGA), represent the major compounds, followed by anthocyanins and flavan-3-ols. High-anthocyanin varieties have high levels of CGA. Both metabolite and transcript levels were higher in skin than in flesh and showed a good correspondence. Two hydroxycinnamoyl-CoA transferases (HCT/HQT) have been involved in CGA production, of which *HCT* reflects CGA levels. Catechin was found in pigmented tissues whereas epicatechin was restricted to tuber skin. Transcripts of leucoanthocyanidin reductase (LCR), which generates catechin, could not be detected. Anthocyanidin reductase (ANR) transcripts, the enzyme responsible for epicatechin production, showed similar levels among samples. These data suggest that the biosynthesis of flavan-3-ols in potato tuber would require ANR but not LCR and that an epimerization process is involved.

1. Introduction

Phenolic compounds are plant-specialized metabolites involved in a wide range of functions. These include (I) pigmentation for flowers, fruits and seeds to attract pollinators and seed dispersers; (II) protection against ultraviolet light; (III) defense against pathogenic microorganisms; and (IV) signaling in plant microbe interactions (Koes, Quattrocchio, & Mol, 1994). Several human health-promoting and therapeutic effects have been reported (Miller, 1996). Many of these effects result from their powerful antioxidant and free-radical-scavenging properties (Rice-Evans, Miller, & Paganga, 1997). Chlorogenic acid (CGA), anthocyanins and flavan-3-ols have been reported to protect against cancer, diabetes and cardiovascular diseases (Scalbert, Manach, Morand, Rémésy, & Jiménez, 2005).

Commercial potato (*Solanum tuberosum* L.), one of the most important food crops (http://faostat3.fao.org/browse/rankings/commodities_by_regions/E), is a good source of CGA and also caffeic acid (CA). Compared with white- and yellow-fleshed commercial potato varieties, red- and purple-fleshed Andean potatoes contain decidedly higher amounts of antioxidant phenolic compounds. This is largely, but not solely, due to anthocyanin biosynthesis (Navarre, Pillai, Shakya, & Holden, 2011). Other flavonoids, such as, flavan-3-ols (catechin and epicatechin), flavanones (eriodictyol and naringenin) and flavonols (kaempferol and quercetin) have been reported in potato tubers (Brown, 2005).

A simplified model of the phenolic compounds metabolism organized in a number of pathways is shown in Fig. 1A. An initial 'core phenylpropanoid pathway' involves phenylalanine-ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H) and 4-coumarate-CoA ligase (4CL), resulting in *p*-coumaroyl-CoA, which is the general precursor of both phenolic acid and flavonoid metabolism (Fig. 1A). At least two routes for the production of CGA, the major phenolic acid found in potato tubers, have been proposed and involve the action of one or two hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferases (HQT/HCT). Route I requires the activity of either HCT or HQT; route II requires the activity of both HCT and HQT (Fig. 1B). (Niggeweg, Michael, & Martin, 2004). Recently we showed that CGA production in

Abbreviations: qRT-PCR, quantitative real time PCR; HPLC-DAD, High-Performance Liquid Chromatography with Diode-Array Detection; CGA, chlorogenic acid; CA, caffeic acid; PAL, phenylalanine-ammonia-lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate-CoA ligase; HCT, hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyltransferase; C3H, p-coumarate 3′-hydroxilase; HQT, hydroxycinnamoyl-CoA quinate hydroxycinnamoyl transferase; CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; DFR, dihydroflavonol 4-reductase; ANS, anthocyanidin synthase; LCR, leucoanthocyanidin reductase; ANR, anthocyanidin reductase.

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http://dx.doi.org/10.1016/j.foodchem.2017.02.150
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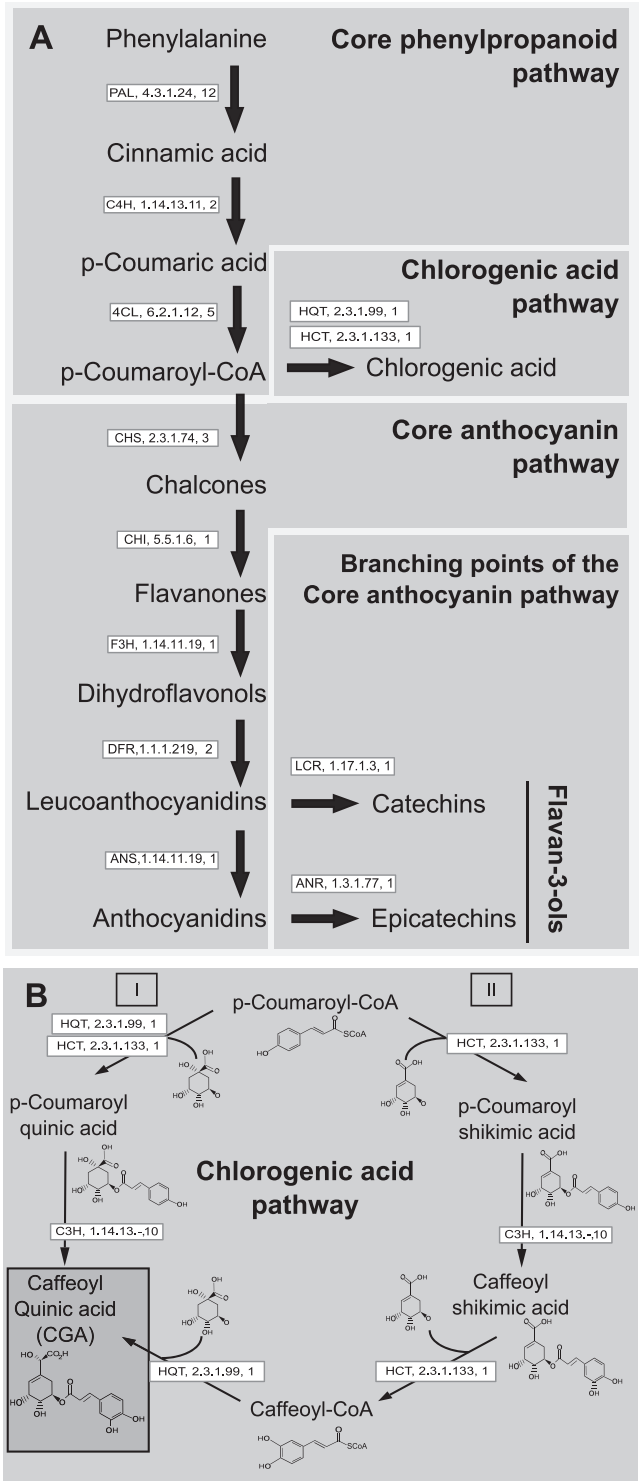

Fig. 1. (A) Schematic diagram of phenolic compounds biosynthetic pathways in plants. (B) CGA biosynthetic pathways in plants. I and II indicate possible routes toward CGA. The abbreviated enzyme names are boxed and followed by the corresponding EC number and the number of possible isoenzymes identified in the potato genome. Abbreviations: PAL, phenylalanine-ammonia lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate-CoA ligase; HCT, hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyltransferase; C3H, *p*-coumarate 3′-hydroxylase; HQT, hydroxycinnamoyl-CoA quinate hydroxycinnamoyl transferase; CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; DFR, dihydroflavonol 4-reductase; ANS, anthocyanidin synthase; LCR, leucoanthocyanidin reductase; ANR, anthocyanidin reductase.

processing potato tubers is likely determined by HQT, rather than HCT (Valiñas, Lanteri, ten Have, & Andreu, 2015).

There are a wide range of chemical structures of anthocyanins due to terminal modifications, including glycosylation, acylation, methylation, etc. However, only six anthocyanidins, sugar-free counterparts of anthocyanins, occur in nature. The production of anthocyanidins is the result of the consecutive action of five enzymes of the 'core anthocyanin pathway' (Fig. 1A) (Schijlen, Ric de Vos, van Tunen, & Bovy, 2004). Besides anthocyanins, other groups of flavonoids branch from the 'core anthocyanin pathway'. Flavan-3-ols can be synthesized either from leucoanthocyanidins by the action of leucoanthocyanidin reductase (LCR or LAR) (Tanner et al., 2003) or from anthocyanidins by anthocyanidin reductase (ANR) (Xie, Sharma, Paiva, Ferreira, & Dixon, 2003). The former produces 2R,3S-*trans*-flavan-3-ols [(+)-catechins] and the latter 2R,3R-*cis*-flavan-3-ols [(−)-epicatechins] (Fig. 1A). In addition, ANR has been reported to possess isomerase activity in some plant species (Pang et al., 2013). Despite the fact that catechin and epicatechin have been reported in potato tubers (Lewis, Walker, & Lancaster, 1998), there are no studies regarding flavan-3-ol biosynthesis yet. The complexity of the metabolic network is increased by the fact that most of the involved enzymes have more than one isoform in potato and that many of the enzymes can have more than one substrate. It is unknown which isoforms are tuber-specific or if isoforms have particular substrate specificity. The assembly of cooperating enzymes into multicatalytic complexes, called metabolons, offers the potential to channel highly reactive or toxic intermediates between active sites and enhance the specificity and efficiency of biochemical pathways (Winkel, 2009). However, there is no evidence yet for this kind of regulation, particularly in potato.

There is a consensus that plant phenylpropanoid biosynthesis is subject of transcriptional rather than post-translational control (Mol, Grotewold, & Koes, 1998). From the plant model *Arabidopsis thaliana* to plant crops such as potato, it was found that the expression of structural genes is coordinately regulated and well correlated with metabolite pools, suggesting that the biosynthesis of phenolic compounds is controlled at the transcriptional level (Yonekura-Sakakibara et al., 2008; André et al., 2009; Stushnoff et al., 2010; Payyavula, Navarre, Kuhl, Pantoja, & Pillai, 2012; Valiñas et al., 2015) and is therefore also subject to metabolic regulation. Strategies to increase the phenolic content in potato included the overexpression of either structural genes or transcription factors. The single-gene overexpression or simultaneous expression of structural genes of the 'core anthocyanin pathway' (Lukaszewicz et al., 2004) or a tuber-specific MYB transcription factor (Rommens et al., 2008) resulted in a significant increase of not only anthocyanins but also phenolic acids in potato tubers. Altogether, data demonstrate that source and sink are important factors but also that changes in a specific flux of phenolic compounds biosynthesis affect other fluxes. Thus, to what extent a specific branch can be increased without unwanted side effects is not well understood and, hence, requires a more delicate analysis. Knowledge of involved fluxes and how are they controlled is required in order to properly design plant breeding programs directed at the increase of desired phenolic compound levels. Separate analysis of flesh and skin tissues is necessary, since metabolite transport has been suggested (Valiñas et al., 2015). Here, we examined the biosynthesis of CGA, anthocyanins and flavan-3-ols through a comparative analysis of flesh and skin tissues of four carefully selected Andean potato varieties taking advantage of their natural variation.

2. Materials and methods

2.1. Plant material

Fifty Andean varieties of *Solanum tuberosum* ssp. *andigena* were grown in fields located in Quebrada de Humahuaca, Jujuy, Argentina during the 2010/2011 season. All varieties were planted on the same date in random plots and harvested at the end of their respective cycles. Nine varieties were selected based on their skin and flesh color for further analysis. For each variety, skin and flesh from ten freshly harvested tubers were pooled to generate a representative sample. The material was immediately frozen in liquid nitrogen and stored at −80 °C until analysis.

2.2. Extraction and quantification of total phenolic acid, flavan-3-ol, anthocyanin, and antioxidant activity

Total phenolic acid levels were determined using the Folin-Ciocalteu method (Singleton, Rossi, & Jr, 1965) with minor modifications (Valiñas et al., 2015). Total phenolic acid contents were expressed as μg of chlorogenic acid equivalents g^{-1} fresh weight (FW).

Flavan-3-ols content was determined according to Chirinos et al. (2007) with minor modifications. Briefly, 200 mg of sample were homogenized with 3 mL of aqueous acetone solvent (70% *v*/*v*) for 2 min. Then the mixture was shaken at 500 rpm in an orbital shaker for 1 h, and centrifuged at 4000*g* for 20 min at 4 °C. The supernatant was collected and concentrated on a rotary evaporator at <35 °C until acetone was eliminated. One milliliter of *p*-dimethylaminocinnamaldehyde (DMACA) solution (250 mg DMACA in 500 mL of HCl:MeOH, 30:70 *v*/*v*) was added to 200 μL of concentrated extract to react at room temperature. Simultaneously, a blank was prepared with distilled water and treated in the same way as the samples. The absorbance was measured at 640 nm. Catechin was used as standard for the calibration curve and the flavan-3-ols content was expressed as μg of catechin equivalents g^{-1} FW.

Anthocyanins were extracted with 1% HCl in methanol. Monomeric anthocyanin content was determined using a pH-differential method (Wrolstad, Culbertson, Cornwell, & Mattick, 1982). A Hitachi U-1900 spectrophotometer and 1-cm pathlength cells were used for spectral measurements at 530 and 700 nm. Pigment content was expressed as μg cyanidin-3-glucoside (Cyd-3-glu) g^{-1} FW, using an extinction coefficient of 34,300 L cm^{-1} mol^{-1} and molecular weight (MW) of 449.2 g mol^{-1}.

Antioxidant activity was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay described by Reddivari, Hale, and Miller (2007) with some modifications (Valiñas et al., 2015). The results were expressed as μg of Trolox equivalents g^{-1} FW.

For all determinations, three independent extractions from a pool of ten tubers were done.

2.3. Phenolic acid and flavonoid analysis

The extraction of phenolic compounds was performed as previously described (Valiñas et al., 2015). Quantification of phenolic compounds was carried out using an Agilent 1100 HPLC system. A flow rate of 0.5 mL min^{-1} was used and 10 μL samples were injected onto a C-18 Phenomenex Luna column (250 × 3 mm i.d.; 5 μm particle size) at 25 °C. The mobile phases were (A) HPLC-grade water acidified with 4% H_3PO_4 and (B) acetonitrile. The solvent program was as follows: 0 to 25 min, gradient from 10% to 25% B, then linear 30% B until 36.5 min, return to 10% B between 36.5 to 37 min and linear at 10% B until 40 min. Simultaneous monitoring was set at 280 nm (catechin and epicatechin), 320 nm (chlorogenic and caffeic acids) and 510 nm (anthocyanidins) for quantification. Anthocyanidins present in the samples were determined after acid hydrolysis, according to the procedure proposed by Durst and Wrolstad (2001). Phenolic acids and flavan-3-ols were quantified in non-hydrolyzed extracts. The external standard method of calibration and peak areas were used for quantitation. Phenolic compound levels were expressed in μg g^{-1} FW.

2.4. Primer design, RNA extraction, cDNA synthesis and qRT-PCR experiments

RNA was extracted from tuber samples using the CTAB (cetyltrimethylammonium bromide) method (Li, Wang, Sun, & Li, 2011). cDNA was synthesized using 2 μg total RNA, previously treated with RNase-free DNase I (Invitrogen, Carlsbad, CA), anchored oligo(dT) 15 VN primers and MMuLV reverse transcriptase according to the manufacturer's description (Invitrogen).

Identification of the phenolic acid biosynthetic genes *PAL*, *C4H*, *4CL*, *HCT*, *C3H*, *HQT*, *CHS*, *CHI*, *F3H*, *DFR*, *ANS*, *LCR* and *ANR* and primer design (sequences in Supplementary Table S1) was carried out following the procedure previously reported (Valiñas et al., 2015).

Relative transcript levels were determined by qRT-PCR in a 10 μL reaction volume with 20 ng RNA equivalent cDNA, 300 nM gene-specific primers and 5 μL SYBR Green Mix (Roche, Mannheim, Germany). Amplification was done using StepOne™ Real-Time PCR System according to the manufacturer's description (Applied Biosystems, Foster City, CA). Relative expression was calculated by the ΔCT method (Livak & Schmittgen, 2001) by normalizing the CT levels of target genes to the geometric mean of CT levels of both housekeeping genes *ACTIN* and cytoplasmic ribosomal protein *L2*. qRT-PCR data represent mean ± SD values from two independent plates of amplification with two wells per cDNA. cDNA was produced from one RNA extraction from a pool of ten potato tubers.

2.5. Statistical analysis

Pearson product moment correlation coefficients (*r* values) were calculated with Microsoft Excel using the means of metabolite concentrations. Differences in the content of metabolites between flesh and skin were evaluated with *t*-test. Previously, normality and homoscedasticity of the data set were tested using the Kolmogorov-Smirnov and Cochran tests, respectively (Zar, 2010).

3. Results

3.1. Selection of Andean potato varieties with different phenolic compound profiles

With the aim to study aspects that determine the levels of CGA, anthocyanins and flavan-3-ols it is necessary to have a group of potato varieties with contrasting profiles of those compounds. Therefore, nine Andean potato varieties, initially selected based on color of skin and flesh (Fig. 2A), were subjected to determinations of total phenolic acid, flavan-3-ol and anthocyanin content in flesh and skin of tubers (Fig. 2B–E). In general, the levels of all phenolic compound groups were higher in skin than in flesh. Phenolic acids was the most abundant group, followed by anthocyanins and flavan-3-ols (Fig. 2B–E). A high qualitative and quantitative variation in phenolic compounds among the screened Andean potato varieties was observed. Chaqueña, Santa María, Waicha and Moradita varieties were selected according to their contrasting profiles of phenolic compounds for further in-depth analysis. The Santa María variety is intensely colored and has by far the highest levels of all compounds in both flesh and skin

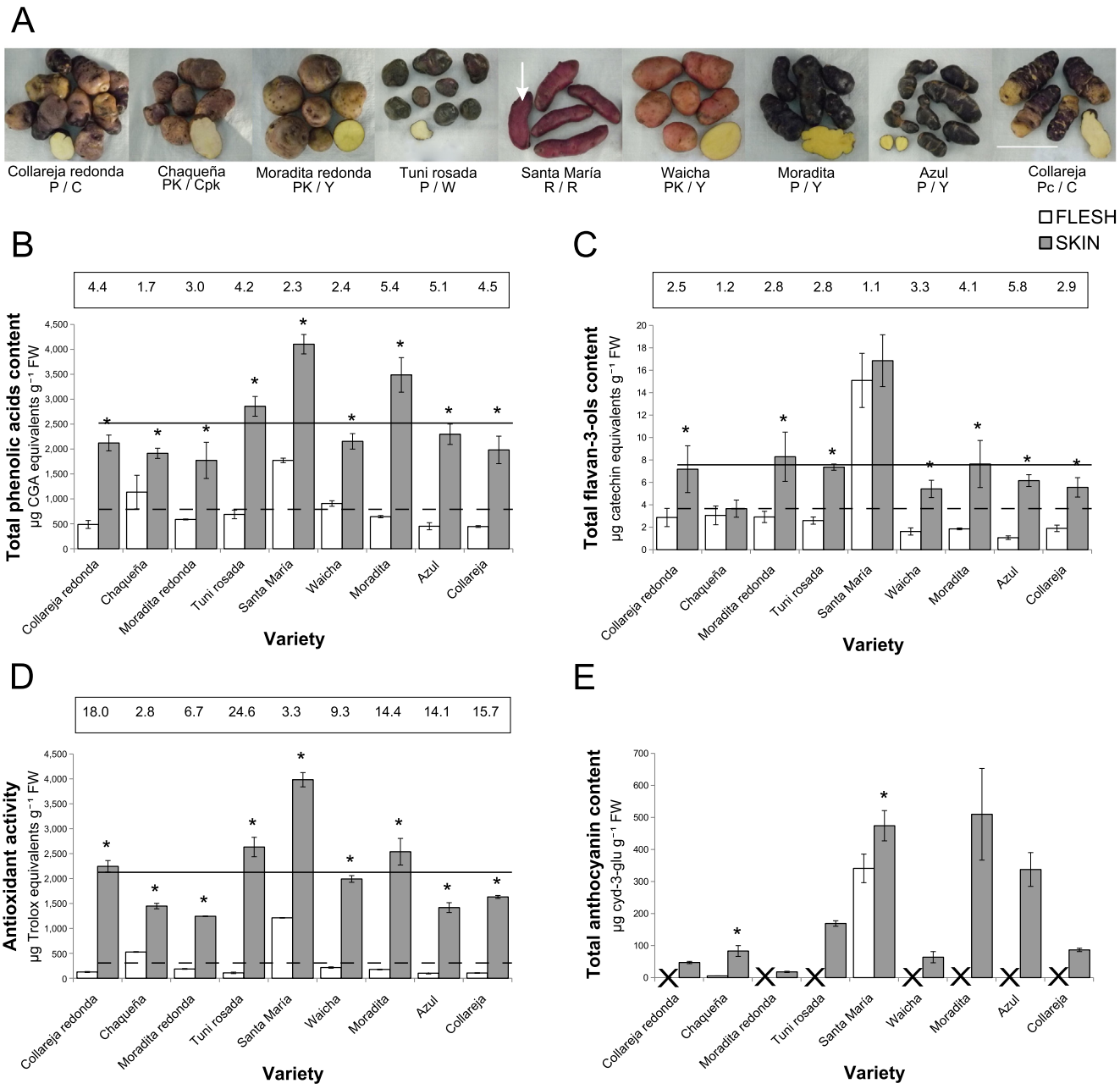

Fig. 2. (A) Tubers from the nine native Andean potato varieties studied. Scale bar = 10 cm. Shown are entire tuber as well as cross-section. The arrow indicates the cut tuber of Santa María variety. Primary (in capital) and secondary skin color; primary (in capital) and secondary flesh color are indicated. P, purple; R, red; PK, pink; Y, yellow; C, cream and W, white (B-E) Total phenolic acids (B), flavan-3-ols (C) and anthocyanins (E) content and total hydrophilic antioxidant activity (D) in flesh (white bars) and skin (grey bars) of tubers from nine Andean potato varieties. Values are presented as the mean ± SD of three independent extractions. Dashed and dash dotted lines indicate the mean values considering the nine varieties in flesh and skin, respectively. Numbers in the upper boxes represent the skin to flesh ratios. X = not detected. Asterisk denotes significant differences between flesh and skin by Student's *t*-test ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fig. 2). Importantly, it is the only variety with significant amounts of anthocyanins in flesh; Chaqueña showing low but still quantifiable levels (Fig. 2E). Chaqueña and Santa María were the varieties with the highest phenolic acid and flavan-3-ol levels in flesh (Fig. 2B and C). In addition, these varieties exhibited the lowest skin-to-flesh ratios of phenolic acids and flavan-3-ols (Fig. 2B and C). The Moradita variety lacks anthocyanins in the flesh but has comparable levels to Santa María in the skin (Fig. 2E). Furthermore, since Moradita and Santa María have purple and red skins, respectively (Fig. 2A), it was expected they would show differences in their anthocyanin composition. Among the non-pigmented flesh varieties, Waicha showed the highest level of total phenolic acids (Fig. 2B). Waicha and Moradita have intermediate and high skin-to-flesh ratios of both phenolic acids and flavan-3-ols, respectively (Fig. 2B and C).

Antioxidant activity was higher in skin than in flesh (Fig. 2D) and a positive correlation was found between phenolic acids and antioxidant activity levels both in flesh and skin ($r = 0.96$ for flesh; $r = 0.91$ for skin). Similarly, a positive correlation was found between phenolic acids and flavan-3-ols ($r = 0.87$ for flesh; $r = 0.78$ for skin). Since antioxidant activity was relatively low in flesh, except for pigmented-flesh varieties Chaqueña and Santa María, it seems that anthocyanins contribute significantly to total antioxidant activity.

3.2. High-anthocyanin varieties have high levels of CGA in both flesh and skin

The levels of the major phenolic acids (CGA, CA), flavan-3-ols (catechin and epicatechin) and anthocyanidins (delphinidin, cyanidin, petunidin, pelargonidin, peonidin and malvidin) in flesh and skin of tubers of the four selected potato varieties were quantified by HPLC-DAD. CGA is the major phenolic acid in flesh and skin of potato tubers (≥80.3%), CA represents up to 19.7% only in pigmented tissues (Table 1). In accordance with the initial screening, content of individual phenolic compounds was higher in skin than in flesh. For example, CGA levels were 4.9 (Chaqueña) to 72.7 (Moradita) times more abundant in skin than in flesh, as indicated by the skin-to-flesh ratio (Table 1). Catechin was only found in pigmented tissues. Epicatechin was detected in the skin of Chaqueña, Santa María and Moradita varieties and contributed at most with 18.6% of total flavan-3-ols (Table 1).

HPLC-DAD profiling of the methanolic potato extracts revealed that several HPLC peaks showed their highest absorbance at 510 nm (Supplementary Fig. S1, upper panel), which is characteristic for anthocyanins. On the basis of the UV–Vis spectra of the anthocyanins, absorption in the 400 460 nm and 310 nm regions indicates the presence of glycosidic substitutions and of acylation with hydroxylated aromatic organic acids, respectively (Hong & Wrolstad, 1990) (see spectral profiles of representative major peaks in Supplementary Fig. S1, upper panel). Because the anthocyanin spectrum of the samples is likely complex and for certain unknown, it would be impractical to try and quantify each peak with its specific standard. Acid hydrolysis of the extract will remove sugar and acyl groups from the various anthocyanins and result in up to six anthocyanidins that occur in nature (Durst & Wrolstad, 2001). This allows for a more feasible analysis when the major interest is in metabolic flows. In accordance with the flesh or skin color, different patterns of anthocyanidins were found in potato methanolic hydrolyzed extracts (Supplementary Fig. S1, lower panel; Table 1). The red skins of Waicha and Santa María varieties contained predominantly pelargonidin (86.9–92.2%) and peonidin (10.8–14%), while the purple-skinned Moradita variety had predominantly petunidin (72.3%), malvidin (13.5%) and peonidin (9.2%), as well as trace amounts of the other anthocyanidins. The skin of Chaqueña contained malvidin (45.5%), pelargonidin (29.7%), peonidin (15.4%) and petunidin (8.1%), whereas the flesh that showed pinkish spots (see Fig. 2A) contained mostly pelargonidin and some peonidin. As expected, none of the six anthocyanidins were detected in non-pigmented flesh of Waicha and Moradita varieties, even though anthocyanidins in skin were detected at rather significant levels (42.7 and 27.2% of the total content of phenolic compounds, respectively; Table 1).

Phenolic acids represent the major phenolic compounds (54.1–100%) followed by anthocyanidins (0–42.7%) in Chaqueña, Waicha and Moradita, except for the flesh of Santa María variety which showed 31% phenolic acids and 65.4% anthocyanidins. Flavan-3-ols represent between 0 and 3.7% of total phenolic compounds. More importantly, all high-anthocyanin varieties also have high CGA levels (Table 1).

3.3. Expression analysis of structural genes of phenolic compound biosynthesis

3.3.1. Hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyltransferase (HCT) reflects CGA levels

Transcript levels of a number of structural genes were quantified by qRT-PCR in flesh and skin of tubers of the four selected Andean potato varieties, in order to explore the molecular mechanisms that determine the levels of CGA, anthocyanins and flavan-3-ols. Genes were selected that have up to three isoforms; genes

Table 1
Phenolic compounds content in flesh and skin of fresh tubers of four Andean potato varieties selected. Data represent the mean ± SD from three independent extractions. Metabolite levels were determined by HPLC-DAD and expressed as μg g^{-1} FW. Numbers in parentheses indicate the percentage of each metabolite with respect to the corresponding total. R indicates skin-to-flesh ratio. ND: not detected

Variety	Chaqueña			Santa María			Waicha			Moradita		
Compound	Flesh	Skin	R	Flesh	Skin	R	Flesh	Skin	R	Flesh	Skin	R
Chlorogenic acid	24.1 ± 0.55 (98.1)	119 ± 7.83 (87.3)	4.9	73.4 ± 5.28 (95.8)	497 ± 33.4 (93.5)	6.8	4.14 ± 0.23 (100)	52.4 ± 3.40 (80.3)	12.7	3.49 ± 0.24 (100)	253 ± 30.4 (86.7)	72.7
Caffeic acid	0.46 ± 0.10 (1.9)	17.2 ± 0.95 (12.7)	37.7	3.21 ± 0.31 (4.2)	34.8 ± 3.17 (6.5)	10.8	ND –	12.9 ± 0.79 (19.7)	–	ND –	38.77 ± 1.37 (13.3)	–
Total phenolic acids	24.5 ± 0.57 (71.9)	136 ± 7.84 (75.2)	5.5	76.6 ± 5.44 (31.0)	531 ± 33.5 (58.5)	6.9	4.14 ± 0.23 (100)	65.2 ± 3.03 (54.1)	15.8	3.49 ± 0.24 (100)	292 ± 29.4 (71.4)	83.8
Catechin	1.28 ± 0.01 (100)	6.17 ± 1.07 (91.7)	4.8	8.97 ± 1.17 (100)	20.0 ± 3.10 (87.9)	2.2	ND –	3.80 ± 0.27 (100)	–	ND –	4.46 ± 0.52 (81.4)	–
Epicatechin	ND –	0.56 ± 0.18 (8.3)	–	ND –	2.74 ± 0.08 (12.1)	–	ND –	ND –	–	ND –	1.02 ± 0.12 (18.6)	–
Total flavan-3-ols	1.28 ± 0.01 (3.7)	6.73 ± 0.89 (3.7)	5.3	8.97 ± 1.17 (3.6)	22.8 ± 3.17 (2.5)	2.5	0 –	3.80 ± 0.27 (3.2)	–	0 –	5.48 ± 0.56 (1.3)	–
Delphinidin	ND –	ND –	–	ND –	ND –	–	ND –	ND –	–	ND –	2.08 ± 0.44 (1.9)	–
Cyanidin	ND –	0.46 ± 0.01 (1.2)	–	0.91 ± 0.30 (0.6)	3.01 ± 0.43 (0.8)	3.3	ND –	1.14 ± 0.11 (2.2)	–	ND –	2.72 ± 0.59 (2.4)	–
Petunidin	ND –	3.09 ± 0.29 (8.1)	–	ND –	ND –	–	ND –	ND –	–	ND –	80.8 ± 16.15 (72.3)	–
Pelargonidin	7.65 ± 1.43 (92.2)	11.2 ± 1.41 (29.7)	1.5	146 ± 23.3 (90.5)	302 ± 27.38 (85.1)	2.1	ND –	44.81 ± 6.79 (86.9)	–	ND –	0.76 ± 0.32 (0.7)	–
Peonidin	0.64 ± 0.01 (7.8)	5.80 ± 0.59 (15.4)	9.1	8.71 ± 3.34 (5.3)	49.9 ± 9.72 (14.0)	5.7	ND –	5.55 ± 0.37 (10.8)	–	ND –	10.3 ± 2.36 (9.2)	–
Malvidin	ND –	17.46 ± 5.56 (45.5)	–	5.80 ± 0.97 (3.6)	ND –	0.0	ND –	ND –	–	ND –	14.7 ± 2.14 (13.5)	–
Total anthocyanidins	8.29 ± 1.45 (24.3)	38.0 ± 3.83 (21.0)	4.6	161.34 ± 27.89 (65.4)	355 ± 37.5 (39.0)	2.2	0 –	51.5 ± 7.27 (42.7)	–	0 –	111 ± 17.7 (27.2)	–

with more isoforms (12 for *PAL* and 5 for *4CL*; see Fig. 1A) were not included in the analysis. Isoform-specific primers were designed for *CH4*, *CHS* and *DFR* (see Supplementary Table S1). The 'core phenylpropanoid pathway' involves the consecutive action of PAL, C4H, and 4CL, of which the two *C4H* isoforms were studied. Transcript levels of *C4H2* were one order of magnitude higher than *C4H1* and, as metabolite levels, were higher in skin than in flesh. Additionally, *C4H2* transcripts levels showed a good correspondence with total phenolic compound levels in flesh but not in skin (Fig. 3A and Table 1). Of the two key enzymes involved in CGA metabolism, *HCT* showed a better correspondence with CGA levels than *HQT* both in flesh and skin of Chaqueña, Santa María and Waicha despite the low levels of expression in all varieties and tissues (Fig. 3B and Table 1). *HQT* levels were significantly higher in the skin of Moradita variety, which also showed high levels of CGA (Fig. 3B and Table 1), suggesting that in this particular variety HQT could be, at least in part, responsible for CGA production.

3.3.2. High-anthocyanin varieties have high expression levels of structural genes of the 'core anthocyanin pathway'

The 'core anthocyanin pathway' leads to the production of anthocyanidins through the consecutive action of five enzymes (CHS, CHI, F3H, DFR, and ANS). In general, transcript levels of structural genes of the 'core anthocyanin pathway' were higher in skin than in flesh, except for *CHI* (Fig. 3C). Transcript levels of *CHS1*, *F3H*, *DFR1* and *ANS* were significantly higher in flesh of the high-anthocyanidin Santa María variety than in non-pigmented flesh (Waicha and Moradita) or low-anthocyanidin content (Chaqueña) varieties. A similar trend was observed in the skin even though fold differences between varieties were lower than in flesh. Curiously, the purple skin of the Moradita variety showed lower transcripts levels of the abovementioned structural genes than would be expected taking into account the metabolite levels observed (Fig. 3C and Table 1).

3.3.3. Flavan-3-ol biosynthesis appears to occur via anthocyanidin reductase (ANR) but not leucoanthocyanidin reductase (LCR)

Flavan-3-ol biosynthesis can occur via two reactions, catalyzed by LCR and ANR, to produce (+)-catechin and (−)-epicatechin, respectively. Despite the fact that catechin was present in pigmented tissues, *LCR* transcripts could not be detected (Supplementary Fig. S2). On the other hand, *ANR* transcripts were present in all samples and showed similar levels between tissues and varieties, and were one to two orders of magnitude lower than the 'core anthocyanin pathway' genes (Fig. 3D).

4. Discussion

The present work was directed to study the biosynthesis of the major antioxidant phenolics CGA, anthocyanins and flavan-3-ols found in potato tubers. A selection of four from nine varieties, made on the basis of total phenolic acid, flavan-3-ol and anthocyanin contents, was used to determine transcript and metabolite levels in flesh and skin tissues. The levels of the groups of compounds determined using the colorimetric assays are in accordance with previous reports for pigmented potato tubers. In accordance with the literature, pigmented tissues contain almost twice the amount of phenolic acids than non-pigmented ones (Navarre et al., 2011).

HPLC-DAD analysis indicates that CGA is the major phenolic acid in both skin and flesh; CA contributed significantly in pigmented tissues. This is in agreement with other reports showing that CGA is the most abundant phenolic acid in potatoes, followed by CA (Navarre et al., 2011; Deusser, Guignard, Hoffmann, & Evers, 2012). Phenolic acids levels estimated by HPLC-DAD showed lower values compared with Folin-Ciocalteu assay. Compounds like thiol derivatives, vitamins, and amino acids may interact with the Folin-Ciocalteu assay, resulting in an overestimation of total phenolic acid levels (Singleton et al., 1965). However, both methods show a good correspondence. Therefore, Folin-Ciocalteu assay remains a rapid and economic method for initial screenings. In accordance with the literature, major anthocyanidins found in red fleshed/skinned tubers were pelargonidin, followed by peonidin, whereas purple potatoes contained petunidin followed by malvidin (Brown, 2005). Similar to what was previously reported, CGA is higher in skin than in flesh (Deusser et al., 2012). Interestingly, epicatechin was restricted to tuber skin, as reported by Lewis et al. (1998).

The biosynthesis of phenolic compounds is mainly controlled at the transcriptional level. In order to visualize associations between metabolite and transcript datasets, data were plotted using radar charts (Fig. 4). Metabolite and transcript levels in skin depict a similar pattern between varieties. The only apparent discrepancy is the absence of epicatechin in Waicha, whereas all transcripts appear present (Fig. 4A). In flesh, where correlations are less clear, Santa María shows, not surprisingly, the highest levels of most compounds and transcripts. Striking is the pattern shown by Waicha and Moradita, the flesh of which lacks anthocyanins and flavan-3-ols but moreover has no detectable CA despite the high levels detected in skin (Fig. 4B). In conclusion, a coordinated expression of the structural genes determines the production of phenolic acids and flavonoids in skin but not in flesh.

Anthocyanin biosynthesis in skin is well explained by the expression levels of structural genes of the 'core anthocyanin pathway' (see Fig. 4A). Basal transcript levels of all 'core anthocyanin pathway' genes were detected in the flesh of both low anthocyanin content variety Chaqueña and white-fleshed Waicha and Moradita varieties. Therefore, the absence of anthocyanins in the flesh cannot not be completely explained by the low transcript levels. We hypothesize this is caused by other fluxes, either down- or upstream. Accordingly, *ANR* transcripts were detected in non-pigmented tissues, indicating an active flavan-3-ol pathway. Therefore, the colored pigment anthocyanidins might be converted to colorless flavan-3-ols by ANR, as soon as they are formed. Although consensus that phenolic compounds are synthesized in a tissue-specific way, they might also be transported (Petrussa et al., 2013) for instance from flesh to skin, thereby providing a voluminous tissue for synthesis of compounds required for stress defenses in the skin. Therefore, the absence of certain metabolites could be additionally explained by metabolite transport. This is particularly clear for CA which represents 19.7 and 13.3% of total phenolic acids in skin of Waicha and Moradita varieties, respectively, but was not detectable in flesh. Epicatechin was only found in skin, in spite of both catechin and *ANR* transcripts being found in the flesh of all pigmented varieties. Previously, we suggested that metabolite contribution from flesh toward skin could be involved in CGA biosynthesis (Valiñas et al., 2015). Hence, although to the best of our knowledge, there are no studies regarding metabolite transport in potato tubers, metabolite contribution from flesh toward skin should be taken into consideration. Further research should be done, in order to determine whether metabolite transport occurs in potato tubers.

HQT was selected as the enzyme responsible for CGA synthesis in Solanaceae species, since overexpression of tomato or tobacco *HQT* in tomato plants resulted in increased levels of thus metabolite whereas silenced plants showed a significant reduction (Niggeweg et al., 2004). Recently, Payyavula et al. (2015) showed that RNAi suppression of *HQT* in potato plants also resulted in over 90% reduction in CGA levels in potato tubers. Accordingly, we recently reported that *HQT* transcript levels reflected CGA levels in processing potato tubers (Valiñas et al., 2015). However, it is still

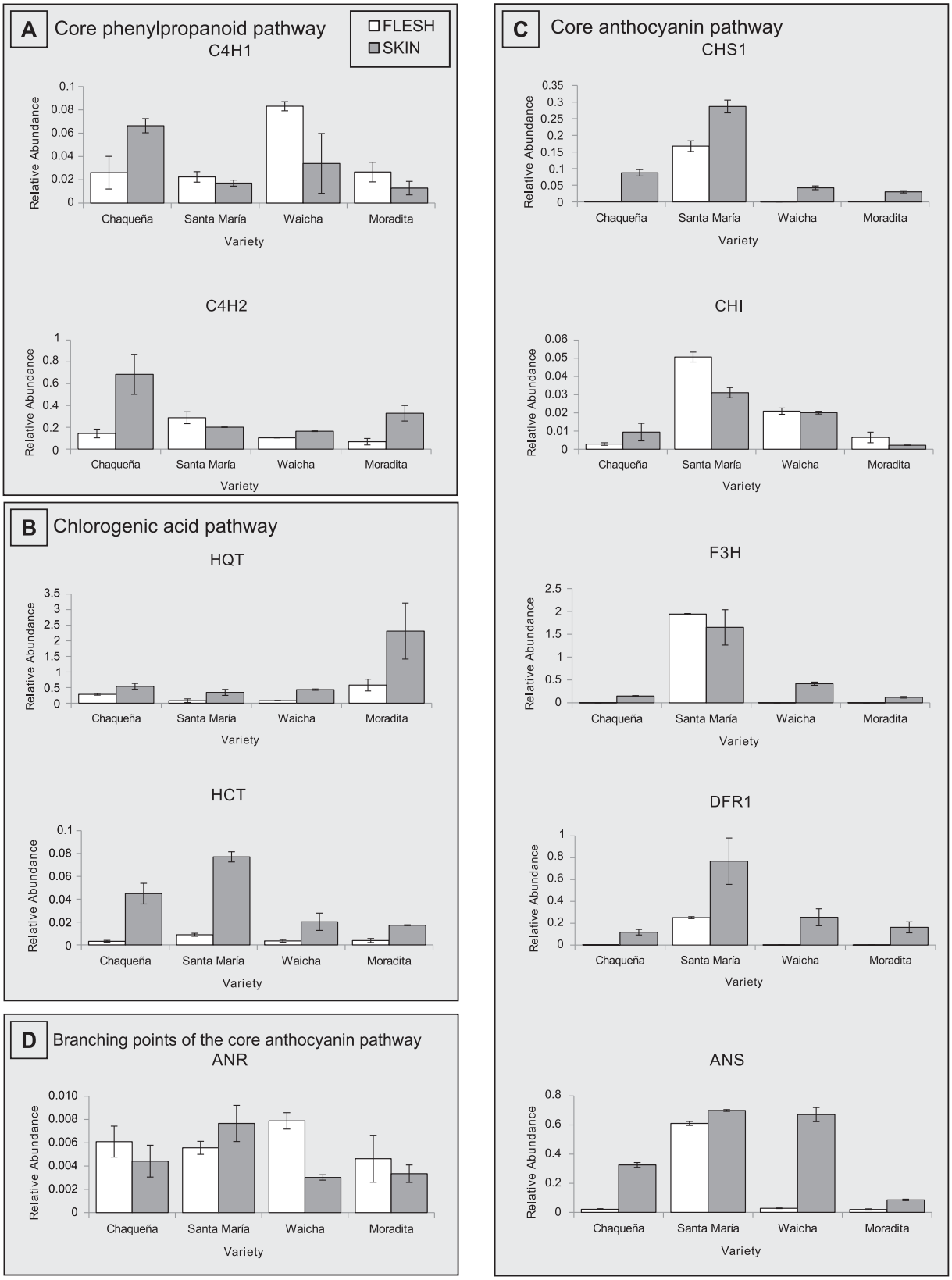

Fig. 3. Transcript levels of phenolic compound biosynthetic genes by qRT-PCR in flesh (white bars) and skin (grey bars) of fresh tubers from four selected Andean potato varieties. qRT-PCR data represent mean ± SD values from two independent plates of amplification with two wells per cDNA.

unclear whether HQT works directly on caffeoyl-CoA and quinic acid to produce CGA (route II; see Fig. 1B) or whether HQT synthesizes *p*-coumaroyl quinate from *p*-coumaroyl-CoA and quinic acid, which is then converted to CGA by the activity of *p*-coumarate 3′-hydroxylase (route I; see Fig. 1B). In the present study, *HQT* does not correspond well with CGA, as is usually the case, even in the

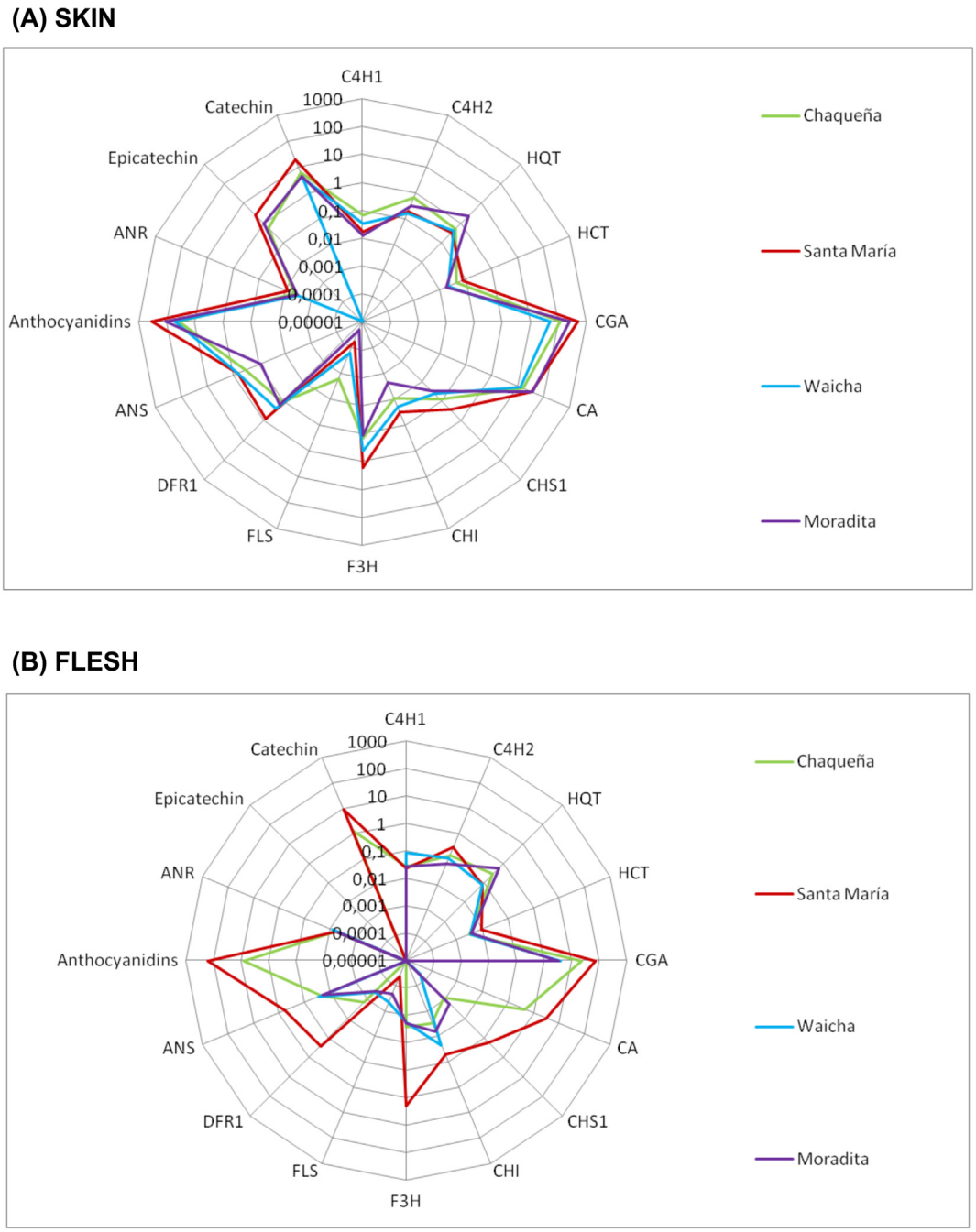

Fig. 4. Relative abundance values of transcript and metabolite levels in μg g^{1} FW of flesh and skin of the four Andean potato varieties characterized with cobweb chart. Axes are in logarithmic scale. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

high-accumulating-CGA cultivar Santa María where a very high expression level of HCT was observed. Other studies carried out in potatoes with varying amounts of CGA due to development, drought or other environmental cues also found a better correspondence between transcript levels of *HCT* and CGA rather than *HQT* (André et al., 2009; Payyavula et al., 2012; Navarre, Payyavula, Shakya, Knowles, & Pillai, 2013). Therefore and in general, route I is likely involved in CGA production *via* HQT where a compensation mechanism could allow the CGA synthesis *via* HCT, in spite of its preference for shikimic acid over quinic acid (Hoffmann, Maury, Martz, Geoffroy, & Legrand, 2003), thereby providing metabolic flexibility. Alternatively Santa María or Andean HCT might have different substrate specificity. The isolation and characterization of potato HCT and HQT would be required to determine their substrate preferences. On the other hand, the lack of correspondence between *HQT* and CGA levels could be explained by its dual catalytic activity recently reported in tomato (Moglia et al., 2014). It has been shown to synthesize dichlorogenate (diCGA) at low pH and high CGA concentration, conditions that occur in vacuoles of plant cells. Accordingly, HQT was shown to localize in vacuoles as well as cytoplasm, supporting the idea that this enzyme catalyzes different reactions in two subcellular compartments. The histidine residue conserved among HQT sequences of Solanaceae species (including potato) essential for this chlorogenate:chlorogenate activity, together with the presence of diCGAs found in potato tubers (Narváez-Cuenca, Vincken, Zheng, & Gruppen, 2013) suggest that HQT from potato would also be able to catalyze this reaction. It is unknown whether HQT synthesizes

CGA and diCGAs simultaneously or first synthesizes CGA and then diCGAs. Navarre et al. (2013) found that CGA levels decrease during tuber development while diCGA levels increase. This result suggests that both events could be taking place simultaneously and might explain why HQT levels do not reflect CGA levels.

Flavan-3-ol production can result from two branching points of the 'core anthocyanin pathway': 1) conversion of leucoanthocyanidins into (+)-catechins by LCR; 2) conversion of anthocyanidins into (−)-epicatechins by ANR. Catechin was detected only in pigmented tissues and its levels were well correlated with anthocyanidin levels. However, *LCR* transcripts could not be detected. Epicatechin was detected only in tuber skin whereas *ANR* transcripts were found in all samples. Taken together, these results suggest that the biosynthesis of flavan-3-ols in potato tuber would occur via ANR but not LCR. Recently, Zhou et al. (2015) demonstrated that in *Prunus persica* the *PpMYB7* gene activates transcription of *PpLCR* but not *PpANR*. Interestingly, the authors checked the publicly available PLAZA database and found that some plant species, among which potato, lack this ortholog. This fact could explain the absence of *LCR* transcripts in potato tubers. The presence of both catechin and epicatechin, suggest that epimerization either chemical (Xie et al., 2003) or via an ANR with epimerase activity (Gargouri et al., 2010; Pang et al., 2013) occurs. It has been reported that high temperatures and alkalization induce epimerization of epicatechin into catechin (Kofink, Papagiannopoulos, & Galensa, 2007). However, this was not the case in the present study, since phenolic extractions were conducted under acidic conditions. Moreover, the accumulated catechin, assumed to be formed through chemical epimerization is observed as a minor by-product (Xie et al., 2003). Here, the amount of catechin accumulated in tubers was higher than epicatechin and was even present in tissues where epicatechin was not found. Thus, the formation of catechin cannot be fully explained by chemical epimerization alone. It was demonstrated that ANR from *Vitis vinifera* and *Camellia sinensis* has intrinsic epimerase activity (Gargouri et al., 2010; Pang et al., 2013). Whether potato ANR has intrinsic epimerase activity or directly convert anthocyanidins into catechin remains to be investigated. The isolation and characterization both *in vitro* and *in vivo* of potato ANR should be done in order to elucidate this matter.

5. Conclusion

The results presented in this work show that phenolic biosynthesis is mainly controlled at the transcriptional level in potato tubers. More importantly, the biosynthesis of flavan-3-ols, a branching point of the 'core anthocyanin pathway', was characterized for the first time in this staple crop. Interestingly, we found that the biosynthesis of both catechin and epicatechin would require ANR but not LCR. Further research should be directed to study the fine tuning regulation of ANR, in order to reroute the biosynthesis to desired compounds. The accumulation of specific metabolites will result in potatoes with beneficial effects in human health.

Acknowledgments

We thank Hernán Bondino for primer design used in qRT-PCR assay. We also thank Andrea Clausen, Ariana Digilio (Banco de Germoplasma INTA-Balcarce, Buenos Aires, Argentina) and Patricia Suárez for assistance in providing plant material. This work was financially supported by grants from Agencia Nacional de Promoción Científica y Tecnológica (PICT 2010 N° 511 and PICT 2010 N° 1812); Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) (PIP N° 0226) and Universidad Nacional de Mar del Plata (UNMdP) (EXA 539/11). A.B.A., A.T.H and M.L.L. are career members from CONICET and M.A.V. is a PhD fellow from CONICET, Argentina.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.02.150.

References

André, C. M., Schafleitner, R., Legay, S., Lefèvre, I., Aliaga, C. A., Nomberto, G., ... Evers, D. (2009). Gene expression changes related to the production of phenolic compounds in potato tubers grown under drought stress. *Phytochemistry, 70*(9), 1107–1116.

Brown, C. R. (2005). Antioxidants in potato. *American Journal of Potato Research, 82*(2), 163–172.

Chirinos, R., Campos, D., Arbizu, C., Rogez, H., Rees, J. F., Larondelle, Y., ... Cisneros-Zevallos, L. (2007). Effect of genotype, maturity stage and post-harvest storage on phenolic compounds, carotenoid content and antioxidant capacity, of Andean mashua tubers (*Tropaeolum tuberosum* Ruiz & Pavón). *Journal of the Science of Food and Agriculture, 87*(3), 437–446.

Deusser, H., Guignard, C., Hoffmann, L., & Evers, D. (2012). Polyphenol and glycoalkaloid contents in potato cultivars grown in Luxembourg. *Food Chemistry, 135*(4), 2814–2824.

Durst, R., & Wrolstad, R. (2001). Separation and characterization of anthocyanins by HPLC. *Current Protocols in Food Analytical*, 1–13.

Gargouri, M., Chaudiére, J., Manigand, C., Maugé, C., Bathany, K., Schmitter, J. M., & Gallois, B. (2010). The epimerase activity of anthocyanidin reductase from Vitis vinifera and its regiospecific hydride transfers. *Biological Chemistry, 391*(2–3), 219–227.

Hoffmann, L., Maury, S., Martz, F., Geoffroy, P., & Legrand, M. (2003). Purification, cloning, and properties of an acyltransferase controlling shikimate and quinate ester intermediates in phenylpropanoid metabolism. *The Journal of Biological Chemistry, 278*(1), 95–103.

Hong, V., & Wrolstad, R. E. (1990). Use of HPLC separation/photodiode array detection for characterization of anthocyanins. *Journal of Agricultural and Food Chemistry, 38*(3), 708–715.

Koes, R. E., Quattrocchio, F., & Mol, J. N. M. (1994). The flavonoid biosynthetic pathway in plants: Function and evolution. *BioEssays, 16*(2), 123–132.

Kofink, M., Papagiannopoulos, M., & Galensa, R. (2007). (-)-Catechin in cocoa and chocolate: Occurence and analysis of an atypical flavan-3-ol enantiomer. *Molecules, 12*(7), 1274–1288.

Lewis, C. E., Walker, J. R. L., & Lancaster, J. E. (1998). Determination of anthocyanins, flavonoids and phenolic acids in potatoes. I: Coloured cultivars of *Solanum tuberosum* L. *Journal of the Science of Food and Agriculture, 77*, 45–57. S. K..

Li, X., Wang, C., Sun, H., & Li, T. (2011). Establishment of the total RNA extraction system for lily bulbs with abundant polysaccharides. *African Journal of Biotechnology, 10*(78), 17907–17915.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods (San Diego, Calif.), 25*(4), 402–408.

Lukaszewicz, M., Matysiak-Kata, I., Skala, J., Fecka, I., Cisowski, W., & Szopa, J. (2004). Antioxidant capacity manipulation in transgenic potato tuber by changes in phenolic compounds content. *Journal of Agricultural and Food Chemistry, 52*, 1526–1533.

Miller, A. L. (1996). Antioxidant flavonoids: Structure, function and clinical usage. *Alternative Medicine Review, 1*(2), 103–111.

Moglia, A., Lanteri, S., Comino, C., Hill, L., Knevitt, D., Cagliero, C., ... Martin, C. (2014). Dual catalytic activity of hydroxycinnamoyl-coenzyme a quinate transferase from tomato allows it to moonlight in the synthesis of both mono- and dicaffeoylquinic acids. *Plant Physiology, 166*(4), 1777–1787.

Mol, J., Grotewold, E., & Koes, R. (1998). How genes paint flowers and seeds. *Trends in Plant Science, 3*(6), 212–217.

Narváez-Cuenca, C. E., Vincken, J. P., Zheng, C., & Gruppen, H. (2013). Diversity of (dihydro) hydroxycinnamic acid conjugates in Colombian potato tubers. *Food Chemistry, 139*(1–4), 1087–1097.

Navarre, D. A., Payyavula, R. S., Shakya, R., Knowles, N. R., & Pillai, S. S. (2013). Changes in potato phenylpropanoid metabolism during tuber development. *Plant Physiology and Biochemistry, 65*, 89–101.

Navarre, D. A., Pillai, S. S., Shakya, R., & Holden, M. J. (2011). HPLC profiling of phenolics in diverse potato genotypes. *Food Chemistry, 127*(1), 34–41.

Niggeweg, R., Michael, A. J., & Martin, C. (2004). Engineering plants with increased levels of the antioxidant chlorogenic acid. *Nature Biotechnology, 22*(6), 746–754.

Pang, Y., Abeysinghe, I. S. B., He, J., He, X., Huhman, D., Mewan, K. M., ... Dixon, R. A. (2013). Functional characterization of proanthocyanidin pathway enzymes from tea and their application for metabolic engineering. *Plant Physiology, 161*(3), 1103–1116.

Payyavula, R. S., Navarre, D. A., Kuhl, J. C., Pantoja, A., & Pillai, S. S. (2012). Differential effects of environment on potato phenylpropanoid and carotenoid expression. *BMC Plant Biology, 12*(1), 39.

Payyavula, R. S., Shakya, R., Sengoda, V. G., Munyaneza, J. E., Swamy, P., & Navarre, D. A. (2015). Synthesis and regulation of chlorogenic acid in potato: Rerouting

phenylpropanoid flux in HQT-silenced lines. *Plant Biotechnology Journal, 13*(4), 551–564.
Petrussa, E., Braidot, E., Zancani, M., Peresson, C., Bertolini, A., Patui, S., & Vianello, A. (2013). Plant flavonoids-biosynthesis, transport and involvement in stress responses. *International Journal of Molecular Sciences, 14*(7), 14950–14973.
Reddivari, L., Hale, A. L., & Miller, J. C. (2007). Determination of phenolic content, composition and their contribution to antioxidant activity in specialty potato selections. *American Journal of Potato Research, 84*(4), 275–282.
Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science, 2*(4), 152–159.
Rommens, C. M., Richael, C. M., Yan, H., Navarre, D. A., Ye, J., Krucker, M., & Swords, K. (2008). Engineered native pathways for high kaempferol and caffeoylquinate production in potato. *Plant Biotechnology Journal, 6*(9), 870–886.
Scalbert, A., Manach, C., Morand, C., Rémésy, C., & Jiménez, L. (2005). Dietary polyphenols and the prevention of diseases. *Critical Reviews in Food Science and Nutrition, 45*(4), 287–306.
Schijlen, E. G. W. M., Ric de Vos, C. H., van Tunen, A. J., & Bovy, A. G. (2004). Modification of flavonoid biosynthesis in crop plants. *Phytochemistry, 65*(19), 2631–2648.
Singleton, V. L., Rossi, J. A., Jr., & Jr, Rossi. J. A. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture, 16*(3), 144–158.
Stushnoff, C., Ducreux, L. J., Hancock, R. D., Hedley, P. E., Holm, D. G., McDougall, G. J., et al. (2010). Flavonoid profiling and transcriptome analysis reveals new gene-metabolite correlations in tubers of *Solanum tuberosum* L. *Journal of Experimental Botany, 61*(4), 1225–1238.
Tanner, G. J., Francki, K. T., Abrahams, S., Watson, J. M., Larkin, P. J., & Ashton, A. R. (2003). Proanthocyanidin biosynthesis in plants. Purification of legume leucoanthocyanidin reductase and molecular cloning of its cDNA. *The Journal of Biological Chemistry, 278*(34), 31647–31656.
Valiñas, M., Lanteri, M., ten Have, A., & Andreu, A. (2015). Chlorogenic acid biosynthesis appears linked with suberin production in potato tuber (*Solanum tuberosum*). *Journal of Agricultural and Food Chemistry, 63*(19), 4902–4913.
Winkel, B. S. J. (2009). Metabolite channeling and multi-enzyme complexes. In A. E. Osborne & V. Lanzotti (Eds.), *Plant-derived natural products – Synthesis, function and application* (pp. 195–208). New York: Springer.
Wrolstad, R. E., Culbertson, J. D., Cornwell, C. J., & Mattick, L. R. (1982). Detection of adulteration in blackberry juice concentrates and wines. *Journal - Association of Official Analytical Chemists, 65*(6), 1417–1423.
Xie, D. Y., Sharma, S. B., Paiva, N. L., Ferreira, D., & Dixon, R. A. (2003). Role of anthocyanidin reductase, encoded by BANYULS in plant flavonoid biosynthesis. *Science, 299*(5605), 396–399.
Yonekura-Sakakibara, K., Toghe, T., Matsuda, F., Nakabayashi, R., Takayama, H., Niida, R., ... Saito, K. (2008). Comprehensive flavonol profiling and transcriptome analysis leading to decoding gene-metabolite correlations in Arabidopsis. *Plant Cell, 20*, 2160–2176.
Zar, J. H. (2010). *Biostatistical analysis*. New Jersey, USA: Prentice Hall.
Zhou, H., Lin-Wang, K., Liao, L., Gu, C., Lu, Z., Allan, A. C., & Han, Y. (2015). Peach MYB7 activates transcription of the proanthocyanidin pathway gene encoding leucoanthocyanidin reductase, but not anthocyanidin reductase. *Frontiers in Plant Science, 6*, 908.