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Phenolic profiles of andean mashua (*Tropaeolum tuberosum* Ruíz & Pavón) tubers: Identification by HPLC-DAD and evaluation of their antioxidant activity

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Abstract

Qualitative high performance liquid chromatography with diode array detection (HPLC-DAD) was performed to characterize non-anthocyanin phenolic compounds in three different coloured mashua genotypes. The ORAC antioxidant activity contribution in the tubers related to the type of phenolic compounds present was also evaluated. Phenolic compounds were analysed by separating them into four main fractions: fraction I obtained by means of a liquid–liquid partition with ethyl acetate and fractions II, III and IV obtained by elution on a Sephadex LH-20 column. Fraction I revealed the presence of gallic acid, gallocatechin, procyanidin B_2 and epigallocatechin. Other phenolic compounds such as hydroxycinnamic and hydroxybenzoic acid derivatives, rutin and/or myricetin derivatives were also present in fraction I. Fraction II was mainly composed of epicatechin, hydroxycinnamic and hydroxybenzoic acid derivatives. Fraction III presented mainly anthocyanins for the purple coloured mashua tubers and rutin, hydroxycinnamic acid and hydroxybenzoic acid derivatives for the yellow coloured genotype. Fraction IV was composed of proanthocyanidins. Alkaline and acid hydrolysis of the different fractions revealed the presence of gallocatechin, epicatechin, *p*-coumaric acid, *o*-coumaric acid, cinnamic acid, protocatechuic acid, rutin and quercetin as the main phenolic moieties present. The proanthocyanidin fractions were the major contributors to the ORAC antioxidant activity of the mashua tubers for two of the three genotypes (34.7–39.2%). The results obtained in the present study confirm that mashua tubers constitute a promising source of antioxidant phenolics and could potentially be considered as a functional food with beneficial health effects.

Keywords: Mashua; *Tropaeolum tuberosum* Ruíz & Pavón; Phenolic profiles; Proanthocyanidins; HPLC-DAD; Sephadex LH-20 chromatography; Liquid–liquid partition; Antioxidant properties

1. Introduction

Mashua (*Tropaeolum tuberosum* Ruíz & Pavón) is an indigenous Andean edible tuber cultivated for centuries from Venezuela to Argentina. Peru is generally believed to be the largest Andean producer. These tubers present a marked diversity in morphology and colour, which varies from cream to dark purple. Mashua diets are reputed to have beneficial health effects. Folk medicinal use of mashua claims, among others, health improvements related to kidney and liver pain, skin eczemas, and prostate disorders (Grau, Nieto, & Hermann, 2003). Health promoting

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properties of mashua tubers may be related to the presence of antioxidant phenolic compounds (Campos et al., 2006; Chirinos et al., 2007).

A recent study aiming at comparing the antioxidant potential of certain Andean crops including coloured potatoes, olluco and oca showed that mashua tubers presented the highest antioxidant activity (Campos et al., 2006). Even more, purple coloured mashua tubers presented eight to ten times higher antioxidant activity than the yellow coloured mashua tubers. This higher antioxidant activity was suspected to be correlated with the high anthocyanin content. Later, Chirinos et al. (2006) found that anthocyanins from mashua were the major contributors to the total antioxidant activity for only one of the three purple mashua tubers, suggesting that other phenolics present are playing a major role in the antioxidant power of mashua tubers. The major anthocyanins found in the different mashua genotypes were delphinidin di- and tri-glycosides acylated with acetic acid. Cyanidins and pelargonidins were also found in minor quantities.

Phenolic compounds are important antioxidants, because of their high redox potentials. They act as reducing agents, hydrogen donors, singlet oxygen quenchers and as metal chelating agents (Tsao & Deng, 2004). Health-related effects of phenolic compounds such as antibacterial (Ezoubeiri et al., 2005; Proestos, Boziaris, Nychas, & Komaitis, 2004), antimutagenic (Pedreschi & Cisneros-Zevallos, 2007), anticarcinogenic (Kähkönen & Heinonen, 2003; Loo, 2003), antithrombotic and vasodilatory activities (Cook & Samman, 1996; Wang, Cao, & Prior, 1997) have been reported. The cited beneficial effects have been related to their antioxidant properties. The number, type and concentration of phenolics in plants exhibit extreme diversity. Phenolic compounds vary in structure: hydroxybenzoic and hydroxycinnamic acids have a single-ring structure while flavonoids comprise three ring structures and can be further classified into anthocyanins, flavan 3-ols, flavones, flavanones and flavonols. Some flavonoids such as flavan 3-ols can be found in the form of dimers, trimers and polymers (Tsao & Deng, 2004). In plants, phenolics mainly occur as glycosylated forms through *o*-glycosidic bonds with a number of different sugars such as glucose, galactose, rhamnose, arabinose, xylose and rutinose (Justesen, Knuthsen, & Leth, 1998). In addition, phenolic compounds also present acylations with phenolic or aliphatic acids, which complicates the identification task.

High performance liquid chromatography (HPLC) is the method of choice for the analysis of phenolic compounds, because of its versatility, precision and relatively low cost (Parejo, Viladomat, Bastida, & Codina, 2004; Robards, 2003). Most frequently, reversed-phase (RP) C_{18} columns, a binary solvent system containing acidified water and a polar organic solvent (acetonitrile or methanol) and UV–Vis diode array detection (DAD) are used and so far constitute a crucial and reliable tool in the routine analysis of plant phenolic compounds (Naczk & Shahidi, 2004). However, the complexity of the phenolic mixtures present in plant materials requires a preliminary clean-up and fractionation of the crude extracts (Prior, Lazarus, Cao, Muccitelli, & Hammerstone, 2004; Robards, 2003;Rodriguez-Saona & Wrolstad, 2001; Sun, Leandro, da Silva, & Spranger, 1998). In addition, phenolic extracts require in many cases a hydrolysis pre-treatment, although, significant phenolic losses may occur due to decomposition of some phenolic compounds (Robards, 2003; Sakakibara, Honda, Nakagawa, Ashida, & Kanazawa, 2003).

To our knowledge, the nature and/or concentration of non-anthocyanin phenolic compounds present in mashua tubers have not been reported yet. Since the phenolic compounds are most likely involved in the health-related properties associated with this tuber, their characterisation is undoubtedly an absolute requirement. In that framework, the objectives of this study were (1) to identify and quantify the main non-anthocyanin phenolic compounds present in three different coloured mashua genotypes using HPLC-DAD and (2) to evaluate the contribution of the different phenolic fractions to the ORAC antioxidant activity of the mashua tubers.

2. Materials and methods

2.1. Sample material

Three different coloured mashua (*Tropaeolum tuberosum*, Ruíz&Pavón) genotypes: ARB 5241 (peel/flesh, purple/yellow), DP 0224 (peel/flesh, purple/purple) and ARB 5576 (peel/flesh, yellow/yellow) were selected with the purpose of evaluating if differences in colour have an influence in their respective phenolic profiles. The genotypes were kindly provided by the International Potato Center (CIP, Lima, Peru) in May 2005. Mashua tubers were harvested at 7.5 months of maturity stage (full maturity). Harvested tubers were immediately lyophilised and stored at −20 °C.

2.2. Reagents and standards

Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid), fluorescein sodium salt, 2 N Folin–Ciocalteu reagent, and *ρ*-dimethylaminocinnamaldehyde (DMACA) were purchased from Sigma Chemicals Co. (St. Louis, MO); 2,2′-aziobis (2-amidinopropane) dihydrochloride (AAPH) was purchased from WAKO Chemicals Industries (Orokama, Japon). Phenolic acids (*p*-coumaric, *o*-coumaric, protocatechuic, ferulic, gallic, caffeic, chlorogenic, *p*-hydroxybenzoic), flavonols (quercetin, rutin, myricetin, kaempherol), flavones (chrysin) and flavanones (naringenin) were purchased from Sigma Chemicals Co. (St. Louis, MO). Flavan 3-ols (cathechin, epicathechin, gallocathechin, epigallocatechin gallate) and procyanidins (procyanidin B1 and B2) were purchased from ChromaDex™ (Santa Clara, CA). Sugar standards (D-glucose, L-rhamnose, D-galactose) and organic acids (malic, fumaric, acetic) were purchased from Sigma Chemicals Co. (St. Louis, MO). HPLC-grade acetonitrile and other solvents and reagents

were purchased from Merck (Darmstadt, Germany). Silica gel 60 F_{254} plates from Merck (Darmstadt, Germany) were used in the separation and identification of sugars by thin layer chromatography.

2.3. Sample preparation

Approximately 5.0 g of lyophilised mashua sample was placed in a screw-cap vial wrapped with aluminum foil and extracted with 300 ml of 0.1% HCl in a solvent mixture (MeOH/acetone/water, 45/45/10, v/v/v). The mixture was vortexed and flushed with nitrogen for 3 min. The vial was allowed to stand in a water bath at room temperature for 60 min under agitation (200 rpm). Then, the extract was centrifuged at 27,000*g* for 10 min at 4 °C and the supernatant was collected. The pellet was submitted to a second extraction of 15 min with 150 ml of solvent mixture. The supernatants were combined and evaporated in a rotary evaporator at 38 °C. The residue was diluted with acidified water (0.01% HCl, v/v) for later phenolic fractionation and identification.

2.4. Fractionation of phenolic extracts

Three different approaches were used for the fractionation of phenolic compounds from mashua: solid-phase extraction (SPE) using a C_{18} Sep-pak cartridge (10 g, 35 cc, Waters, Milford, MA, USA) (Rodriguez-Saona & Wrolstad, 2001), liquid–liquid partition with ethyl acetate (Kennedy, 2002) and Sephadex LH-20 (Sigma, St. Louis, MO) column chromatography (Prior et al., 2004). Twenty five milliliter of extract were passed through a C_{18} Sep-pak cartridge previously activated with methanol and acidified water (0.01% HCl). Phenolics were adsorbed onto the column. Sugars, acids, and other polar compounds were eluted with 70 ml of acidified water (0.01% HCl). The phenolic fraction was then eluted with 100 ml of acidified methanol (0.01% HCl). The methanol was then removed under vacuum (38 °C), and the residue was re-dissolved in 8 ml of Milli-Q water and referred to as purified mashua extract. Six ml of the purified mashua extract were submitted to a partition with 12 ml of ethyl acetate. Partition included a 20 min agitation (200 rpm) under darkness. Then, the mixture was let to stand until two phases were observed and the organic phase was collected. A second partition was performed under the same conditions. The organic phases were assembled and evaporated to dryness. This fraction is referred to as fraction I. The remaining aqueous phase was lyophilised and then dissolved in 6 ml of 20% methanol. Two ml of this obtained extract were further applied on a Sephadex LH-20 column (470 × 15 mm i.d.). Elution was carried out with 120 ml of 20% methanol/water (v/v) followed by 400 ml of 60% methanol/water (v/v) and finally by 600 ml of 60% acetone/water (v/v) at a flow rate of 1 ml/min. This fractionation process was repeated three times. The three eluates were collected separately for antioxidant activity evaluation and phenolics identification. These fractions are referred to as fraction II, fraction III and fraction IV and were concentrated under vacuum (38 °C) and then freeze-dried. At a later stage, part of freeze-dried fractions was re-resuspended in HPLC-grade methanol for HPLC-DAD analysis. All fractions obtained were flushed with nitrogen and were stored at −20 °C until analyses. Total recoveries of phenolic compounds and ORAC antioxidant activity were calculated based on the basis of a mass balance. Percentage recovery was obtained by taking into account the initial phenolic and ORAC values of the sample submitted to the fractionation process and the obtained phenolic and ORAC values in each fraction.

2.5. Alkaline and acid hydrolysis of phenolic fractions

The fractions obtained as described above were saponified following the protocol used by Llorach, Gil-Izquierdo, Ferreres, and Tomás-Barberán (2003). The purpose of this procedure was to release acyl groups potentially present on the phenolic compounds. Hydrolysis was performed by adding 1 ml 4 N NaOH per mg of dried fraction and the mixture was kept for 16 h in a screw-cap test tube, under a nitrogen atmosphere. Concentrated HCl was added (yellow colour change, pH 1.0) to the alkaline hydrolysis product and the resulting mixture was directly analysed by HPLC-DAD.

The fractions were acid-hydrolysed following the protocol proposed by Hertog, Holman, and Venema (1992). A 20 mg sample of dried fraction was hydrolysed using 5 ml of a 50% methanol acidified solution (1.2 M HCl) for 2 h at 90 °C. The solution was then adjusted to a final volume of 15 ml with 50% methanol and was cooled to room temperature. The purpose of this step was to release sugar molecules from the phenolic compounds.

2.6. HPLC-DAD analysis of phenolic compounds

The phenolic compound profiles were determined according to the procedure proposed by Tsao and Yang (2003), with slight modifications. The purified phenolic extracts or fractions, treated or not with acid or basic solutions, as described above, were separated using a reversed-phase HPLC column on a Waters 2695 Separation Module (Waters, Milford, MA) equipped with an autoinjector, a 996 photodiode array detector (DAD) and the Empower software. Spectral data were recorded from 200 to 700 nm during the whole run. An X-terra RP_{18} (5 μm, 250 × 4.6 mm) column (Waters, Milford, MA) and a 4.6 mm × 2.0 mm guard column were used for phenolic separation at 30 °C. The mobile phase was composed of solvent (A) water:acetic acid (94:6, v/v, pH 2.27) and solvent (B) acetonitrile. The solvent gradient was as follows: 0–15% B in 40 min, 15–45% B in 45 min, and 45–100% B in 10 min. A flow rate of 0.5 ml/min was used and 20 μl of sample were injected. Samples and mobile phases were filtered through a 0.22 μm Millipore filter, type GV (Millipore, Bedford, MA) prior to HPLC injection. Each extract/

fraction was analysed in triplicate. Phenolic compounds were identified and quantified by comparing their retention time and UV–visible spectral data to known previously injected standards.

2.7. Study of the hydrolysis products (organic acids and sugars)

The organic acids released after alkaline hydrolysis were determined by HPLC-DAD according to the methodology proposed by Cawthray (2003). Organic acids were separated through reversed-phase HPLC on a Waters 2695 Separation Module equipped with an autoinjector, a 996 photodiode array detector (DAD) and the Empower software. Spectral data from 190 to 400 nm were recorded during the whole run. An Atlantis™ C_{18} (5 μm, 150 × 4.6 mm) column (Waters, Milford, MA) and a 4.6 mm × 2.0 mm guard column were used at 30 °C. The mobile phase consisted of 93% 25 mM KH_2PO_4 adjusted to pH 2.5 with concentrated *ortho*-phosporic acid and 7% methanol. A flow rate of 1 ml/min for 30 min was used and 20 μl of sample were injected. Detection of organic acids was recorded at 210 nm. Each hydrolyzate was analysed in triplicate.

The sugars released after acid hydrolysis were evaluated by qualitative TLC analysis according to the methodology proposed by Toba and Adachi (1978). A small amount (5 μl) of concentrated hydrolysate was applied to the silica gel 60 F_{254} plates. Sugars were separated using propanol:water (85:15, v/v) as solvent. The presence of separated sugars was detected by submerging the prepared TLC plates in diphenylamine–aniline–phosphate revealing solution. Sugars were identified by comparing their Rf values to known sugar standards run in parallel.

2.8. Oxygen radical absorbance capacity (ORAC) assay

The hydrophilic antioxidant activity of the purified mashua extracts and their fractions were determined using the ORAC assay. ORAC analyses were performed in a 96-well microplate fluorometer (Ascent F.L. Fluoroscan, Labsystem, Finland) and were adapted from the procedures described by Ou, Hampsch-Woodill, and Prior (2001) Huang, Ou, Hampsch-Woodill, Flanagan, and Prior (2002). AAPH, a water-soluble azo compound, was used as a peroxyl radical generator. Trolox, a water soluble tocopherol analogue, was used as the standard and fluorescein as a fluorescent probe. Fluorescence filters were used for an excitation wavelength of 485 nm and an emission wavelength of 520 nm. Briefly, 25 μl of PBS buffer pH 7.4 (blank), Trolox standard or diluted sample in PBS buffer at pH 7.4 were mixed with 250 μl of 55 nM fluorescein and incubated for 10 min at 37 °C before automatic injection of 25 μl AAPH solution (153 nM). Fluorescence was measured every minute for 50 min. The final ORAC values were calculated using the net area under the decay curves and were expressed as μmol of Trolox Equivalents (TE)/g mashua fresh weight (FW).

2.9. Determination of total phenolics

Total phenolics in purified mashua extracts and their fractions were determined with the Folin–Ciocalteu reagent by the method of Singleton and Rossi (1965) using gallic acid as a standard. Absorbance was measured at 755 nm and the results were expressed as mg gallic acid equivalents (GAE)/100 g mashua FW.

2.10. Statistical analysis

Quantitative data are presented as mean values with the respective standard deviation values corresponding to three replicates. Antioxidant capacity, total phenolic and total flavan 3-ol results were processed by the one-way analysis of variance (ANOVA). A Duncan test was used to determine significant differences. Differences at $p < 0.05$ were considered as significant. SPSS for Windows 14.0 (SPSS, Chicago, IL, USA) was used for all statistical tests.

3. Results and discussion

3.1. Phenolic profiles of mashua extracts

Phenolic profiles at 280 nm for the three mashua genotypes evaluated are presented in Fig. 1. HPLC-DAD profiles for the purple mashua genotypes ARB 5241 and DP 0224 contained roughly the same type of phenolic compounds, even though differences in concentration of each individual compound were observed (data not shown). In both genotypes, anthocyanins (referred to as "A" in Fig. 1) appeared to be the main phenolics present. The yellow mashua genotype ARB 5576 presented a slightly different phenolic profile in comparison to the purple coloured mashua genotypes. An almost total absence of peaks with retention times from 31 to 50 min (mashua anthocyanin retention time) was observed. Peaks in the retention time range from 51 to 62 min characterised the phenolic profile for this genotype.

We introduced a phenolic fractionation step for the mashua extracts in order to facilitate the identification of the phenolic compounds and to evaluate the ORAC antioxidant activity associated with each fraction. The fractionation method performed yielded four fractions (referred to as fractions I, II, III and IV) which were characterised at 280, 320, 360 and 520 nm to allow a tentative identification of the predominant phenolic compounds (Table 1). The chromatograms obtained at 280 nm for each fraction of the ARB 5241 and ARB 5576 mashua genotypes are shown in Fig. 2a and b, respectively. Data regarding the DP 0224 genotype are not shown, because of their similarity to the ARB 5241 genotype.

3.1.1. Fraction I analysis

Phenolics from fraction I were obtained using ethyl acetate in a liquid–liquid partition. The use of this method has been proposed in many studies (Kennedy, 2002; Krasteva,

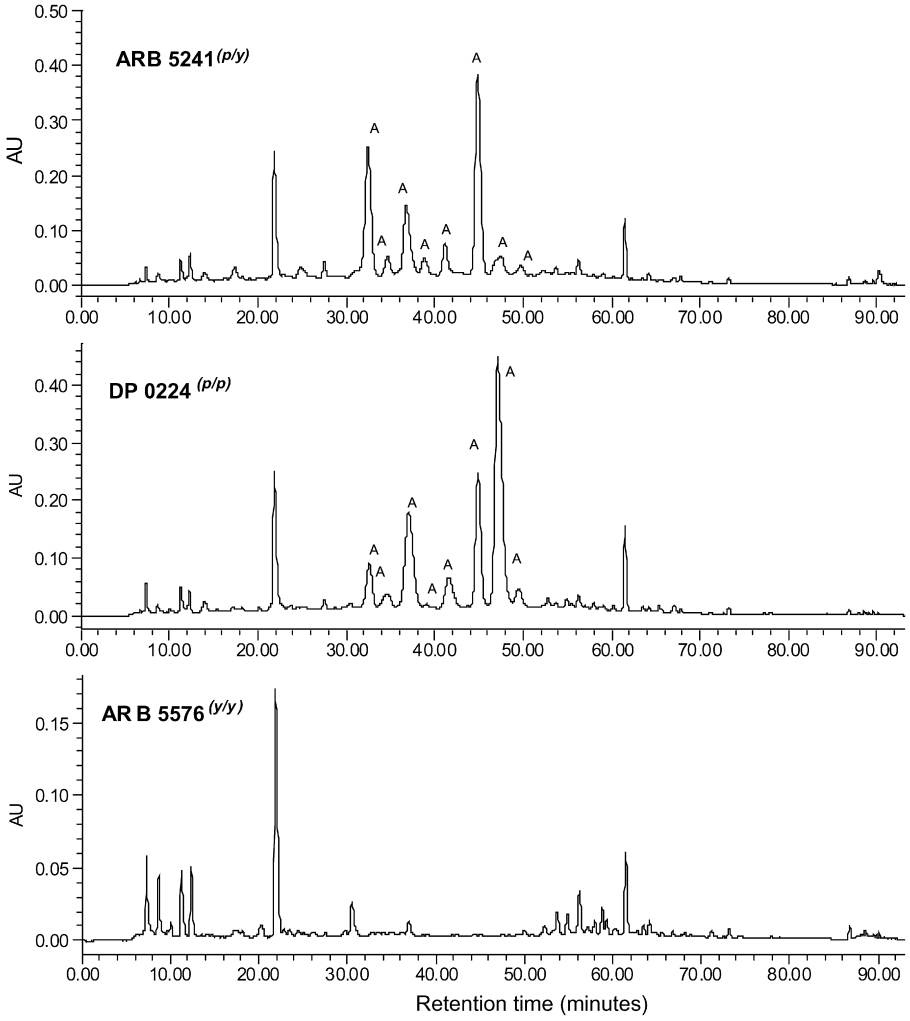

Fig. 1. HPLC-DAD phenolic profiles for the three mashua genotypes recorded at 280 nm. (p/y) purple peel/yellow flesh, (p/p) purple peel/purple flesh, (y/y) yellow peel/yellow flesh. A: anthocyanin.

Nikolova, Danchev, & Nikolov, 2004; Pedreschi & Cisneros-Zevallos, 2007). Krasteva et al. (2004) mentioned that the affinity of flavonols for ethyl acetate is due to their hydrophobic character, whereas Kennedy (2002) pointed out that removal of monomeric flavan 3-ols is possible with liquid–liquid extraction using ethyl acetate. Flavonols and phenolic acids from purple corn were recovered using ethyl acetate (Pedreschi & Cisneros-Zevallos, 2007).

The HPLC chromatograms recorded at 280 nm for fractions I for the ARB 5241 and ARB 5576 mashua genotypes are shown in Fig. 2a and b. In both chromatograms we can distinguish a whole set of different compounds, which were not observed in the initial extracts, possibly due to a co-elution of phenolic compounds during the initial separation. Fractions I contained a variety of different phenolic compounds such, as flavan 3-ols, phenolic acids and flavonols (Table 1). Peaks 1, 2, 5, 7–9, 13, 16–18, 20, 22 and 25–26 were common to all mashua genotypes, whereas peaks 27–36 were present only in the yellow coloured mashua genotype. Peaks identified in fractions I corresponded to gallic acid (peak 1), gallocatechin (peak 2), epigallocatechin (peak 5) and procyanidin B_2 (peak 9). Identification was based on retention times and UV–Vis spectral data compared to previously injected known standards. The major not identified phenolics in this fraction showed UV–Vis spectral characteristics similar to phenolic acids such as *o*-coumaric acid, *p*-coumaric acid, *p*-hydroxybenzoic acid and cinnamic acids, flavan 3-ols such as epicatechin, gallocatechin and epigallocatechin and flavonols such as rutin and myricetin.

In order to obtain additional information on the glycosylation and acylation patterns, basic and acid hydrolysis processes were performed. After alkaline hydrolysis, the chromatographic profiles for fractions I showed that peaks 1, 2, 5, 20 and 24 remained constant, whereas peaks 3, 4, 6–19, 21–23, and 25–27 for the purple coloured mashua genotypes and peaks 7–9, 13, 16–18, 22, 25–37 for the yellow coloured mashua genotype disappeared with the subsequent appearance of new peaks. The new appearing peaks did not correspond to any of the well-known standards but displayed similar UV–Vis spectral characteristics than the initial phenolics found in the fractions I (data not shown). Disappearance of phenolics after basic hydrolysis indicated

Table 1
Chromatographic and spectral characteristics of non-anthocyanin phenolics detected in different mashua tuber fractions by HPLC-DAD[a]

Fraction number	Peak number	Retention time (min)	λ_{max} (nm)	Phenolic compound assignment	Relative area$_{280\ nm}$ (%)[a]	Amount (mg/100 g mashua FW[a])
Fraction I						
ARB 5241$^{(y/y)}$	1	11.9	273	Gallic acid	0.58	0.02
	2	16.5	236, 270	Gallocatechin	1.80	1.26
	3	21.6	236, 280	Epicatechin derivative	0.98	0.12
	4	23.4	236, 280	Epicatechin derivative	2.44	0.29
	5	25.2	237, 270	Epigallocatechin	17.9	10.7
	6	27.8	237, 273	Epigallocatechin derivative	0.99	0.59
	7	30.8	237, 275	Epigallocatechin derivative	2.37	1.42
	8	33.5	237, 270	Gallocatechin derivative	4.72	3.31
	9	34.8	237, 280	Procyanidin B_2	10.8	1.43
	10	41.8	292	Cinnamic acid derivative	10.2	6.73
	11	47.2	284	Cinnamic acid derivative	1.05	1.30
	12	48.7	284, 325	*o*-Coumaric acid derivative	3.98	0.20
	13	51.0	274, 325	*o*-Coumaric acid derivative	1.69	0.08
	14	54.9	273, 320	*o*-Coumaric acid derivative	1.48	0.07
	15	56.7	259, 355	Rutin derivative	0.40	0.02
	16	58.2	287, 325	*o*-Coumaric acid derivative	6.86	0.34
	17	59.0	309	*ρ*-Coumaric acid derivative	1.19	0.08
	18	60.7	289, 316	*o*-Coumaric acid derivative	0.91	0.34
	19	63.1	246, 276	Epicatechin derivative	1.26	0.15
	20	64.3	268	*ρ*-Hydroxybenzoic acid derivative	7.98	6.83
	21	68.1 c	254, 377	Myricetin derivative	1.39	0.03
	22	69.2	236, 280	Epicatechin derivative	1.06	0.13
	23	72.2	310	*ρ*-Coumaric acid derivative	0.76	0.05
	24	76.3	234, 290, 326	Unknown	14.0	0.66
	25	79.0	260, 354	Rutin derivative	0.67	0.04
	26	82.1	247, 280	Epicatechin derivative	1.62	0.19
	27	83.4	279, 325	*o*-Coumaric acid derivative	1.25	0.06
DP 0224$^{(p/p)}$	1	11.8	272	Gallic acid	1.31	0.03
	2	16.4	236, 270	Gallocatechin	Tr	
	3	21.5	235, 279	Epicatechin derivative	Tr	
	4	23.4	235, 279	Epicatechin derivative	1.08	0.09
	5	25.2	235, 270	Epigallocatechin	7.07	3.02
	6	28.0	235, 272	Epigallocatechin derivative	8.48	3.62
	7	30.4	235, 273	Epigallocatechin derivative	1.24	0.53
	8	33.5	236, 270	Gallocatechin derivative	7.45	3.73
	9	34.8	235, 279	Procyanidin B_2	2.61	0.25
	10	41.8	292	Cinnamic acid derivative	9.29	3.27
	11	47.1	284	Cinnamic acid derivative	1.85	1.64
	12	48.8	280, 324	*o*-Coumaric acid derivative	7.40	0.26
	13	51.0	276, 324	*o*-Coumaric acid derivative	3.38	0.12
	14	54.9	272, 322	*o*-Coumaric acid derivative	2.19	0.08
	15	56.7	266, 355	Rutin derivative	0.44	0.02
	16	58.1	283, 325	*o*-Coumaric acid derivative	9.49	0.34
	17	59.0	309	*ρ*-Coumaric acid derivative	0.91	0.05
	18	60.6	282, 320	*o*-Coumaric acid derivative	1.01	0.04
	19	63.1	241, 278	Epicatechin derivative	1.98	0.17
	20	64.3	267	*ρ*-Hydroxybenzoic acid derivative	7.75	4.34
	21	68.1	253, 376	Myricetin derivative	1.90	0.03
	22	69.2	235, 279	Epicatechin derivative	1.79	0.15
	23	72.2	310	*ρ*-Coumaric acid derivative	0.30	0.01
	24	76.3	235, 291, 325	Unknown	11.9	0.42
	25	79.6	259, 354	Rutin derivative	0.90	0.04
	26	82.1	241, 279	Epicatechin derivative	2.80	0.24
	27	83.3	271, 325	*o*-Coumaric acid derivative	1.52	0.05
ARB 5576$^{(y/y)}$						
	1	12.0	275	Gallic acid	2.69	0.01
	2	16.3	238, 270	Gallocatechin	0.54	0.05
	28	23.5	283	Cinnamic acid derivative	0.81	0.18
	5	25.3	237, 270	Epigallocatechin	1.68	0.14
	29	28.0	273, 325	*o*-Coumaric acid derivative	8.76	0.08
	7	30.7	235 275	Epigallocatechin derivative	3.09	0.25
	8	33.7	231, 268	Gallocatechin derivative	10.3	0.97
	9	35.0	235, 278	Procyanidin B_2	0.72	0.01
	30	46.1	235, 277	Epicatechin derivative	2.64	0.04
	31	47.5	294	Cinnamic acid derivative	0.77	0.17
	13	51.2	243, 323	*o*-Coumaric acid derivative	7.8	0.07
	32	55.3	286	Cinnamic acid derivative	4.82	1.09
	33	57.5	281, 321	*o*-Coumaric acid derivative	3.42	0.03

Table 1 (*continued*)

Fraction number	Peak number	Retention time (min)	λ_{max} (nm)	Phenolic compound assignment	Relative area$_{280\ nm}$ (%)[a]	Amount (mg/100 g mashua FW[a])
	16	58.5	271, 325	*o*-Coumaric acid derivative	3.08	0.03
	17	59.2	312	*ρ*-Coumaric acid derivative	3.42	0.04
	18	60.3	278, 313	*o*-Coumaric acid derivative	2.16	0.02
	34	63.5	269	*ρ*-Hydroxybenzoic acid derivative	6.16	0.81
	20	64.6	268	*ρ*-Hydroxybenzoic acid derivative	5.93	0.77
	22	69.5	236, 281	Epicatechin derivative	3.98	0.06
	35	73.0	268	*ρ*-Hydroxybenzoic acid derivative	1.22	0.16
	24	76.7	244, 290, 327	Unknown	11.1	0.10
	25	78.1	259, 370	Rutin derivative	1.58	0.02
	36	79.9	259, 369	Rutin derivative	1.69	0.02
	26	82.4	236, 280	Epicatechin derivative	2.21	0.04
	37	83.6	241, 277	Epicatechin derivative	1.31	0.02
Fraction II						
ARB 5241	1	11.5	273	Gallic acid	5.23	0.43
	1′	17.8	284	Cinnamic acid derivative	1.86	0.26
	2′	18.2	294	*ρ*-Coumaric acid derivative	1.63	0.10
	3′	22.0	231, 280	Epicatechin derivative	65.8	16.6
	4′	52.1	235, 280	Epicatechin derivative	Tr	
	5′	61.0	253, 276	Protocatechuic or vanillic acid derivative[b]	23.6	3.65
	6′	63.0	252, 276	Protocatechuic or vanillic acid derivative[b]	1.11	0.17
	7′	63.8	278	Cinnamic acid derivative	Tr	
	8′	67.2	255, 272	Protocatechuic or vanillic acid derivative[b]	0.46	0.07
DP 0224	1	11.6	273	Gallic acid	4.71	0.35
	1′	17.8	281	Cinnamic acid derivative	1.45	0.35
	2′	18.2	292	*ρ*-Coumaric acid derivative	2.01	0.08
	3′	22.2	231, 280	Epicatechin derivative	45.9	8.63
	4′	52.1	235, 280	Epicatechin derivative	4.04	0.75
	5′	61.1	254, 273	Protocatechuic or vanillic acid derivative[b]	30.3	4.20
	6′	63.1	252, 277	Protocatechuic or vanillic acid derivative[b]	2.46	0.34
	7′	63.8	278	Cinnamic acid derivative	4.13	0.66
	8′	67.7	236, 278	Protocatechuic or vanillic acid derivative[b]	4.93	0.68
ARB 5576	9′	10.8	274	Gallic acid derivative	17.6	0.33
	1	11.4	272	Gallic acid	20.0	0.38
	1′	17.6	283	Cinnamic acid derivative	2.97	0.02
	2′	18.1	293	*ρ*-Coumaric acid derivative	3.96	0.06
	3′	22.0	235, 280	Epicatechin derivative	17.9	1.14
	10′	51.7	285	Cinnamic acid derivative	3.78	0.07
	11′	54.2	254, 273	Protocatechuic or vanillic acid derivative[b]	2.99	0.10
	5′	69.8	254, 274	Protocatechuic or vanillic acid derivative[b]	21.6	0.75
	12′	62.8	253, 274	Protocatechuic or vanillic acid derivative[b]	2.77	0.10
	7′	63.6	279	Cinnamic acid derivative	4.84	0.04
Fraction III						
ARB 5576	1	11.6	273	Gallic acid	26.6	0.46
	1″	22.3	230, 280	Epicatechin derivative	11.0	5.53
	2″	30.4	294, 321	*o*-Coumaric acid derivative	12.5	1.51
	3″	53.7	251, 277	Protocatechuic acid derivative	5.17	0.17
	4″	55.8	252, 274	Protocatechuic acid derivative	14.3	0.47
	5″	58.2	256, 352	Rutin derivative	6.75	0.53
	6″	58.7	256, 356	Rutin derivative	7.15	0.54
	7″	63.5	256, 355	Rutin derivative	16.5	1.29
Fraction IV						
ARB 5241				Peak detected at the end of the chromatogram		
DP 0224				Peak detected at the end of the chromatogram		
ARB 5576				Peak detected at the end of the chromatogram		

[a] Mean of three repetitions.

[b] Quantified as protocatechuic acid. (p/y) purple peel/yellow flesh, (p/p) purple peel/purple flesh, (y/y) yellow peel/yellow flesh. Tr: Traces. Flavan 3-ols and their derivatives (epigallocatechin, gallocatechin, epicatechin and proanthocyanidin B_2) and hydroxybenzoic acid derivatives (gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid) were quantified at 280 nm using their corresponding standard aglycon. Hydroxycinnamic acid derivatives (*o*-coumaric acid, *ρ*-coumaric acid, cinnamic acid) were quantified at 320 nm using their corresponding standard aglycon. Flavonol derivatives (myricetin and rutin) were quantified at 360 nm using their corresponding standard aglycon. Unknown compounds were quantified as *o*-coumaric acid.

the presence of acylations in their molecular structures. The presence of aliphatic acids (malic, fumaric and acetic) in the different fractions could however not be detected by the HPLC method performed.

The acid hydrolysis showed that peaks 1, 5, 13 and 24 remained constant, whereas peaks 2–4, 6–23 and 25–27 for the purple coloured mashua genotypes and peaks 2, 7–9, 16–18, 20–22, 26–36 for the yellow coloured mashua

genotype disappeared. Based on their UV–Vis spectral data and their retention time, the appearance of gallocatechin, epicatechin, *o*-coumaric acid and quercetin was confirmed for the yellow and purple coloured mashua fractions. Additionally, myricetin, delphinidin and cyanidin were present in the purple coloured mashua fractions. The other produced peaks by hydrolysis could not be identified. Delphinidin and cyanidin appeared as aglycones due to traces of mashua anthocyanins in this fraction. Quercetin could have been formed from rutin (quercetin 3-*o*-rutinoside). Acid hydrolysis indicated that sugar molecules could be attached to phenolics in this fraction. Only the presence of released glucose was confirmed with the qualitative TLC analysis of sugars in all genotypes (data not shown).

On the basis of the results of the acid and alkaline hydrolyses, the different peaks present in fraction I were tentatively identified as gallocatechin derivative (peak 8), epigallocatechin derivatives (peaks 3,4,6,7), epicatechin derivatives (peaks 3,4,19,22,26,29,36), *o*-coumaric acid derivatives (peaks 12–14,16,18,27,28,33), *p*-coumaric derivatives (peaks 17, 23), cinnamic acid derivatives (peaks 10,11,27,30,31), *p*-hydroxybenzoic acid derivatives (peaks 20,33,34) myricetin derivatives (peak 21) and rutin derivatives (peaks 15,25,35). Finally, the total amount of flavan

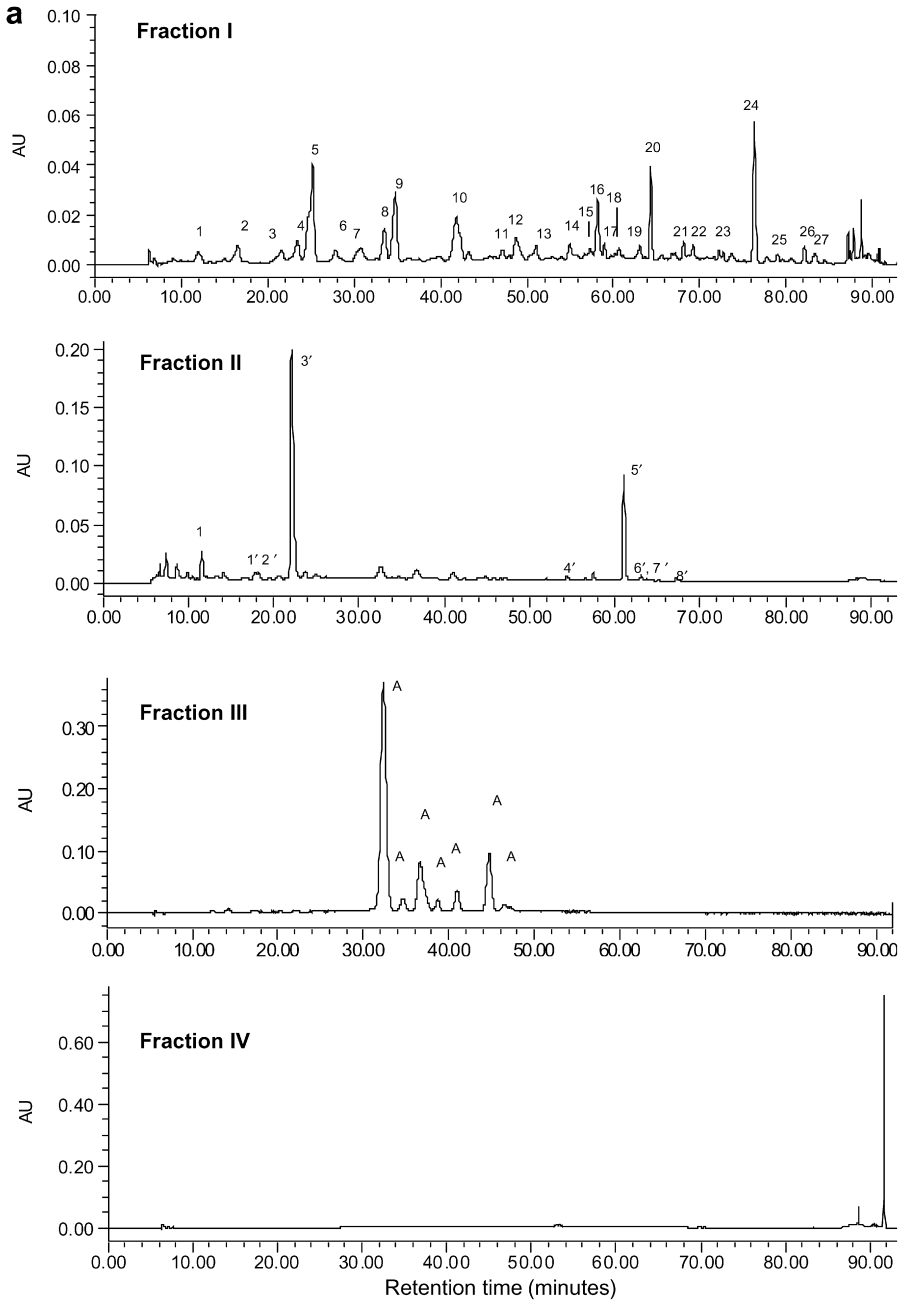

Fig. 2. Chromatographic profiles at 280 nm for fractions I, II, III, and IV obtained from ARB 5241 (a) and ARB 5576 (b) mashua genotypes. A: anthocyanin. The identification and quantification of the different peak numbers is presented in Table 1.

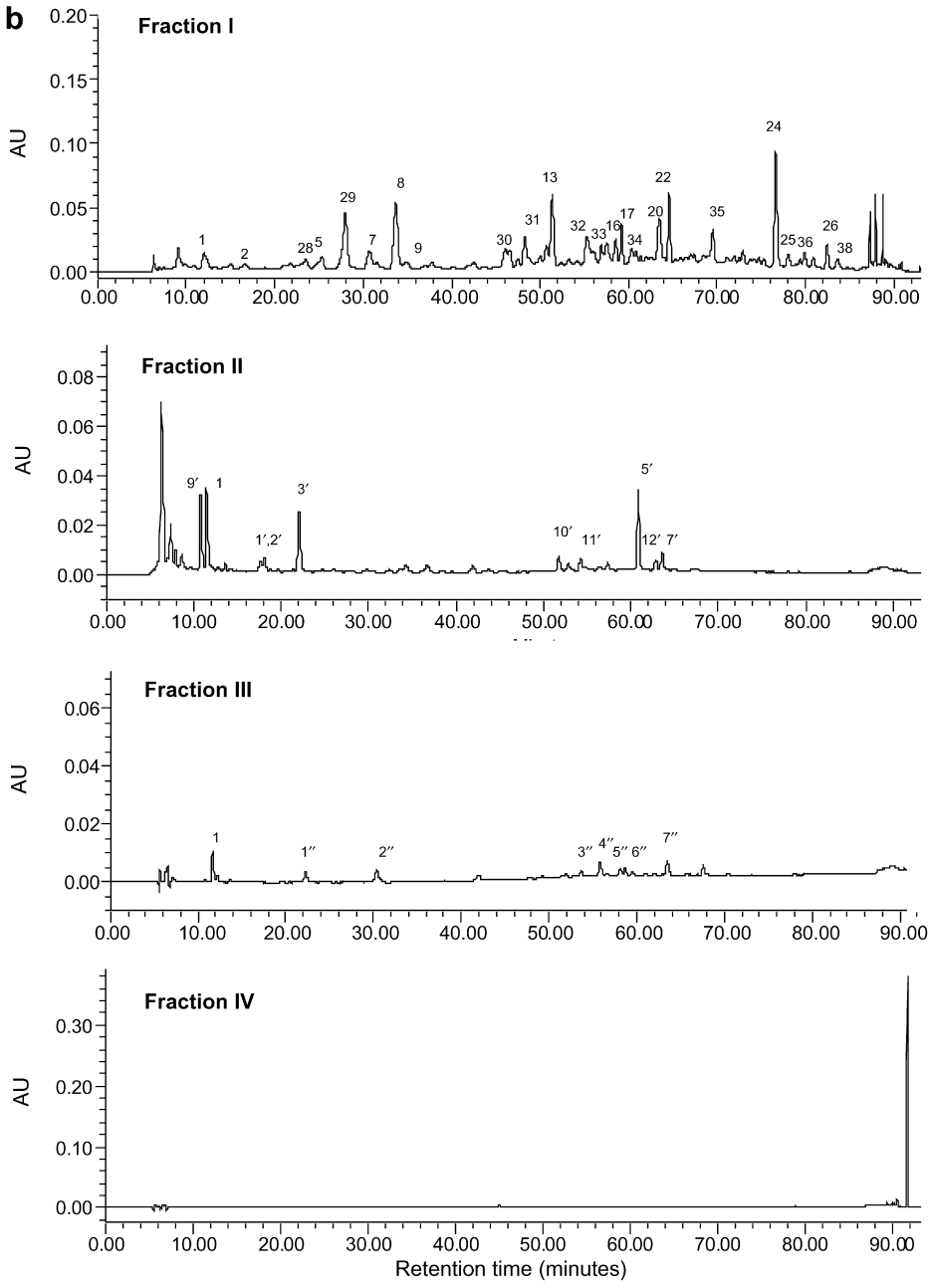

Fig. 2 (*continued*)

3-ols, hydroxycinnamic acids, hydroxybenzoic acids and flavonols for ARB 5241, DP 0224 and ARB 5576 genotypes were ~18.1, 11.5 and 1.5, ~9.2, 5.8 and 1.9, ~6.8, 4.3 and 1.6 and ~0.09, 0.09 and 0.04 mg/100 g FW, respectively (Table 1).

3.1.2. Fraction II analysis

The water-soluble phenolics of mashua tubers were submitted to fractionation by means of a Sephadex LH-20 gel permeation chromatography as described by Chirinos et al. (2006), Kantz and Singleton (1990) and Prior et al. (2004). The purpose of this step was the separation of these phenolic compounds and avoidance of interfering substances in further analyses. Fraction II was obtained by means of an elution with 20% MeOH/water (v/v). Prior et al. (2004) indicated the collection of phenolic acids with this eluent. The HPLC chromatograms obtained at 280 nm for fractions II of the ARB 5241 and ARB 5576 mashua genotypes are shown in Fig. 2a and b. Fractions II were indeed composed mainly by phenolic acids (Table 1). A total of nine (1 and, 1′–8′) and ten (1, 1′–3′, 5′, 7′ and, 9′–12′) representative peaks were found for the purple and yellow coloured mashua genotypes, respectively. Only gallic acid was identified in this fraction for all the genotypes. Small quantities of anthocyanins (A) were eluted in this fraction in the case of the purple coloured mashua geno-

types. Other phenolics in this fraction showed UV–Vis spectral characteristics similar to epicatechin and phenolic acids, such as *p*-coumaric acid, protocatechuic or vanillic acid and cinnamic acid. Vanillic acid has similar UV spectral characteristics to protocatechuic acid and since this standard was not evaluated in this study, there is a possibility of presence of vanillic acid in this fraction. Alkaline hydrolysis showed the disappearance of peaks 1′, 2′, 4′–8′ and appearance of new peaks. Peak 1 (gallic acid) and peak 3′ (epicatechin derivative) remained constant. The new peaks that appeared after the alkaline hydrolysis did not correspond to any of the phenolic standards run but showed UV–Vis spectral characteristics similar to the initial phenolics found in the non-hydrolysed fraction II (data not shown). No aliphatic acid was detected by the HPLC method used.

Acid hydrolysis yielded new peaks. None of the peaks obtained after the acid hydrolysis corresponded to the phenolic standards evaluated. Only gallic acid remained in this fraction and peak 3′ was slightly displaced to the left. The presence of released glucose in fractions II was confirmed with the TLC analysis for all the genotypes (data not shown).

On the basis of the results of the acid and alkaline hydrolyses, the different peaks present in fraction II were tentatively identified as epicatechin derivatives (peaks 3′, 4′), cinnamic acid derivatives (peaks 1′, 7′, 10′), *p*-coumaric acid derivatives (peaks 2′), and protocatechuic or vanillic acid derivatives (peaks 5′, 6′, 8′, 11′, 12′). The total amount of flavan 3-ols, hydroxycinnamic and hydroxybenzoic acids for the ARB 5241, DP 0224 and ARB 5576 genotypes were ~16.6, 9.3 and 1.1, ~0.3, 1.1 and 0.1 and ~4.3, 5.5 and 1.6 mg/100 g FW, respectively (Table 1).

3.1.3. Fraction III analysis

Fraction III was obtained by means of an elution with 60% MeOH/water (v/v) during the sephadex LH-20 gel permeation chromatography. Prior et al. (2004) recovered a high quantity of anthocyanins from blueberry and cranberry (67–100%), as well as chlorogenic acid (92.8%) and rutin (72.0%) with this eluent. The HPLC chromatograms obtained at 280 nm for fractions III corresponding to the ARB 5241 and ARB5576 mashua genotypes are shown in Fig. 2a and b. Fractions III for the purple coloured mashua genotypes were composed mainly by anthocyanins (A). Identification of mashua anthocyanins has been performed in a previous study (Chirinos et al., 2006).

The chromatographic profiles for fraction III of the yellow coloured mashua ARB 5576 genotype indicated the presence of 8 predominant peaks (peaks 1 and 1″–7″). Peak 1 corresponded to gallic acid detected in fraction II. The UV–Vis spectral data for peaks 1″–7″ presented similar characteristics to epicatechin, *o*-coumaric acid, protocatechuic acid and rutin (Table 1). Alkaline hydrolysis revealed the presence of cinnamic acid and a rutin derivative. Peaks 1, 1″ and 2″ remained constant. Acid hydrolysis revealed the presence of protocatechuic acid, quercetin and one peak, which could not be identified. In summary, the different peaks that appeared in fraction III for the yellow coloured mashua genotype were tentatively identified as an epicatechin derivative (peak 1″), an *o*-coumaric acid derivative (peak 2″), protocatechuic acid derivatives (peaks 3″, 4″) and rutin derivatives (peaks 5″–7″). The total amount of flavan 3-ols, hydroxycinnamic and hydroxybenzoic acids and flavonols for ARB 5576 genotype were ~5.5, 1.5, 1.1 and 2.3/100 g FW, respectively (Table 1).

3.1.4. Fraction IV analysis

Recoveries of polymeric polyphenols corresponding to proanthocyanidins have been achieved using Sephadex LH-20 gel permeation chromatography in different studies (Kantz & Singleton, 1990; Kennedy, 2002; Prior et al., 2004; Skrede, Worlstad, & Durst, 2000). In the present work, fraction IV was eluted from the Sephadex LH-20 column with 60% acetone/water (v/v). Under the same conditions, Kantz and Singleton (1990) obtained a procyanidin-rich fraction from grape tissue extracts. Accordingly, procyanidins from blueberry were recovered from the Sephadex LH-20 column by eluting with 70% (v/v) aqueous acetone (Gu et al., 2002; Skrede et al., 2000). The use of 66% aqueous acetone to remove proanthocyanidins from various plant sources using a Toyopearl HW-40F column has also been recommended (Kennedy, 2002).

The chromatographic profiles for fractions IV showed absence of representative peaks between retention times from 10 to 85 min (Fig. 2a and b). A single peak appeared at the end of the chromatograms for all the mashua genotypes, indicating that the compounds of fraction IV are poorly separated by means of reversed-phase HPLC and present thus lypophilic characteristics. Accordingly, Adamson et al. (1999) indicated that the analysis of procyanidins using reversed-phase HPLC with UV detection at 280 nm is ineffective in separating the higher oligomers (>trimer) and as a result these compounds elute as a large unresolved peak. This peak does very likely correspond to a collection of polymeric polyphenols belonging to the proanthocyanidin family. The proanthocyanidins are composed of mixtures of oligomers and polymers containing flavan 3-ol units; among them the most common are the procyanidins, which consist exclusively of (epi)catechin and their gallic esters and of the prodelphinidins, which consist of (epi)gallocatechin and their galloylated derivatives (Gu et al., 2002).

After alkaline hydrolysis of fraction IV, one representative peak (peak 1‴) was obtained for all the genotypes evaluated (Fig. 3a1 and b1). Peak 1‴ could not be identified with any of the phenolic standards evaluated. However, this peak showed UV–Vis spectral data characteristics of the members of the flavan 3-ol phenolic family (λ_{max} 239.4, 275.2 nm). In a previously reported study, it was shown that the conversion of proanthocyanidins from grape skin to known subunits (epigallocatechin, catechin) was associated to a dramatic degradation of proanthocy-

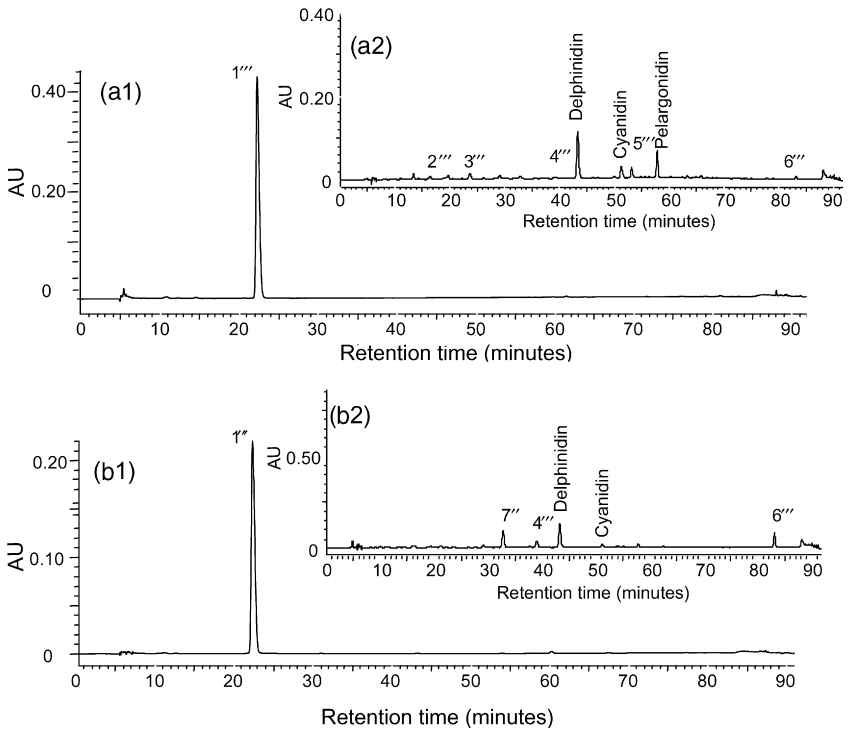

Fig. 3. HPLC-DAD chromatograms for fraction IV for the ARB 5241 and ARB 5576 mashua genotypes obtained after alkaline (a1, b1) and acid (a2, b2) hydrolysis respectively.

anidins over the course of the oxidative basic reaction (Jorgensen, Marin, & Kennedy, 2004).

Acid hydrolysis produced different peaks. In the purple coloured mashua genotype fractions (Fig. 3a2) peak 2‴ corresponded to gallocatechin and peaks 3‴, 4‴ and 5‴ corresponded to derivatives of gallocatechin, catechin and epicatechin. Anthocyanidins, such as delphinidin, cyanidin and pelargonidin, were present, probably because of the hydrolysis of remaining anthocyanins in this fraction or due to the presence of proanthocyanidins. For the yellow coloured mashua genotype, acid hydrolysis also yielded anthocyanidins, such as delphinidin and cyanidin (Fig. 3b2). Transformation of proanthocyanidins to anthocyanidins under acid conditions has already been reported (Porter, Hrtich, & Chan, 1986). Additionally, three peaks (4‴, 6‴ and 7‴) with similar characteristics to epicatechin and catechin were present. In a similar way, Skrede et al. (2000) hydrolysed an isolate of procyanidins from blueberries with 0.01 M HCl for 30 min and the HPLC analysis of the hydrolysate revealed a decrease in procyanidins with a simultaneous increase in catechin and epicatechin.

The results found in the present work suggest that mashua tubers present proanthocyanidins belonging to the family referred to as prodelphinidins and procyanidins. But, this is something that should be further investigated. More studies are necessary to elucidate the structure of this polymeric phenolic fraction. Proanthocyanindin identification by means of normal-phase HPLC with photodiode or fluorescence detection and thiolysis for evaluating their degree of polymerisation (DP), as well as extension units, would be necessary. Finally, phenolic losses and degradation of phenolics could have occurred during the fractionation process or during the hydrolysis process. As an example, flavonoids are very susceptible to hydrolysis (Llorach et al., 2003) due to the decomposition of polyphenols (Sakakibara et al., 2003).

Table 2
Total phenolics (mg gallic acid equivalents/100 g mashua FW, GAE/100 g mashua FW) and ORAC antioxidant activity (μmol Trolox equivalents/g mashua FW, μmol TE/g mashua FW) for the obtained individual mashua fractions after the fractionation process for the purified extracts from three mashua genotypes[a]

Mashua genotype	Total Phenolics (mg GAE/100 g mashua FW)	ORAC antioxidant activity (μmol TE/g mashua FW)
ARB 5241$^{(p/y)}$		
Fraction I	38.4 ± 2.1	6.5 ± 0.6
Fraction II	33.9 ± 2.3	2.7 ± 0.4
Fraction III	126 ± 9.8	17.1 ± 0.9
Fraction IV	161 ± 12.2	21.0 ± 1.0
DP 0224$^{(p/p)}$		
Fraction I	23.5 ± 1.9	4.2 ± 0.3
Fraction II	27.8 ± 1.2	2.6 ± 0.3
Fraction III	145 ± 7.8	17.6 ± 0.7
Fraction IV	40.5 ± 9.4	5.8 ± 0.9
ARB 5576$^{(y/y)}$		
Fraction I	6.7 ± 0.4	1.2 ± 0.1
Fraction II	1.3 ± 0.1	0.2 ± 0.0
Fraction III	13.4 ± 0.1	1.9 ± 0.2
Fraction IV	25.7 ± 2.2	3.1 ± 0.2

[a] $x \pm$ SD of three separate fractionations.

3.2. Total phenolics and ORAC antioxidant activity of mashua fractions

The total phenolic contents were 395, 262 and 52.2 mg GAE/100 g FW, respectively for the ARB 5241, DP 0224 and ARB 5576 mashua genotypes. The purple coloured mashua genotypes presented 5 to 7-fold higher phenolic contents as compared to the yellow coloured mashua genotype. The total phenolic contents for the purple genotypes is in the same range as the contents reported for apples, cherries, plums, strawberry, broccoli and cabbages (211–347, 339, 366, 368, 284–286 and 203–321 mg GAE/100 g FW, respectively) (Wu et al., 2004), but higher than the values found in grapes, beans, onions, potatoes and sweet potatoes (145–175, 96, 74–126, 122–179 and 74–120 mg GAE/100 g FW, respectively) (Wu et al., 2004). Mean ORAC antioxidant activities of 60.5, 37.6 and 7.9 μmol TE/g FW were found for the ARB 5241, DP 0224 and ARB 5576 genotypes, respectively. The highest ORAC value (60.5 μmol TE/g FW) is even higher than the values reported for blackberry, raspberry, strawberry, red cabbage, red potato and sweet potato (52.4, 47.6, 35.4, 14.3, 13.1 and 8.5 μmol TE/g FW, respectively). The highest ORAC values were obtained for the ARB 5241 genotype, the same one that showed the highest total phenolic content.

The obtained values for total phenolics and ORAC antioxidant activity for each fraction after fractionation process of the purified mashua extracts are shown in Table 2. After fractionation, most of the phenolics were present in fractions III (13.4–126.4 mg GAE/100 g FW) and fractions IV (25.7–160.7 mg GAE/100 g FW). Minor quantities of phenolics were found in fractions I and II. Fraction III for genotype DP 0224 and fraction IV for the genotypes ARB 5241 and ARB 5576 presented the highest contribution to the total phenolics (55.4, 40.8 and 49.7%, respectively, Fig. 4a). Since the DP 0224 mashua genotype is the one with the highest anthocyanin content (Campos

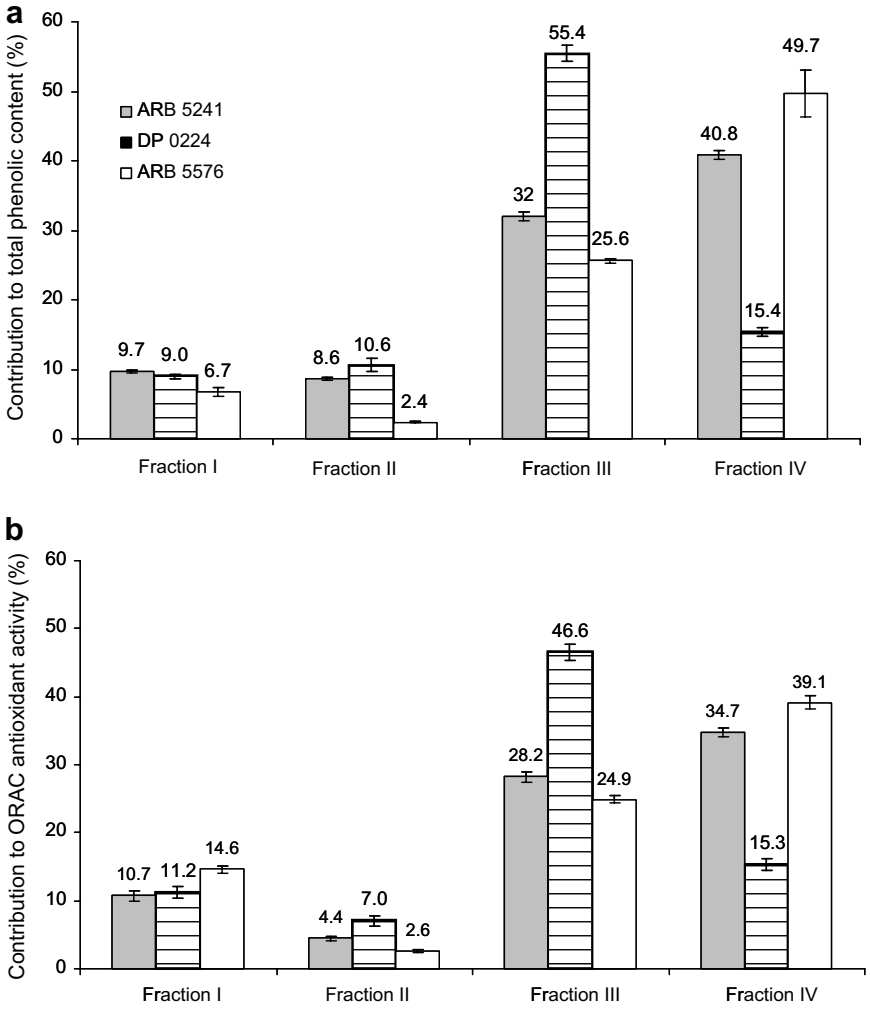

Fig. 4. Phenolic fraction contribution (%) to the total phenolics content (a) and to the total ORAC antioxidant activity (b) in purified mashua tuber. Values above the bars indicate the contribution (%) of the individual phenolic fractions for each one of the evaluated characteristics. Bars indicate mean values ± SD for three replicates.

et al., 2006; Chirinos et al., 2006) and considering that the mashua anthocyanins are mainly recovered by means of a 60% methanol/water mixture applied on the Sephadex LH-20 column (Chirinos et al., 2006), it is very likely that the higher phenolic recovery in fraction III for DP 0224 genotype is related to the high anthocyanin content. The HPLC profile at 280 nm for fraction III (Fig. 2a) showed that anthocyanins are the major phenolics present in this fraction. The presence of proanthocyanidins in fraction IV (HPLC analysis described above) could be related to the highest values of phenolics in this fraction for the genotypes ARB 5241 and ARB 5576.

Fraction IV for the ARB 5576 and ARB 5241 genotypes presented the highest ORAC values (21.0 and 3.1 μmol TE/g FW) followed by fraction III (17.1 and 1.9 μmol TE/g FW), fraction I (6.5 and 1.2 μmol TE/g FW) and finally by fraction II (2.7 and 0.2 μmol TE/g FW) (Table 2). The DP 0224 mashua genotype presented the following order in terms of ORAC values: fraction III (17.6 μmol TE/g FW) > fraction IV (5.8 μmol TE/g FW) > fraction I (4.2 μmol TE/g FW) > fraction II (2.6 μmol TE/g FW). In brief, fraction III for genotype DP 0224 and fraction IV for the genotypes ARB 5241 and ARB 5576 presented the highest contribution to the ORAC antioxidant activity 46.6, 34.7 and 39.1%, respectively (Fig. 4b). Thus, the major contribution to the ORAC antioxidant activity appears to be related to the proanthocyanidin content for the ARB 5241 and ARB 5576 mashua genotypes and to phenolics belonging to the anthocyanin family for the DP 0224 genotype. Interestingly, fraction III appeared as the second best contributor to the ORAC antioxidant activity for the yellow coloured mashua genotype. This finding could be related to the combined presence of flavonols (rutin type), flavan 3-ols (epicatechin type) and phenolic acids (*o*-coumaric, protocatechuic and gallic acid) (Table 1) found in this fraction. Losses during the fractionation process for total phenolics and ORAC values were 8.9–9.7% and 18.2–21.8%, respectively.

The relationship between phenolic content and ORAC antioxidant activity was determined in the four fractions. Fractions I, II, III and IV presented a good correlation coefficient of 0.9983, 0.9801, 0.9891 and 0.9976, respectively, for ORAC values vs. phenolics. These results suggest that phenolic compounds were directly responsible for the ORAC antioxidant activity in all fractions. The ratios of antioxidant activity to phenolics (μmol TE/mg GAE) were in the range 16.9–17.9, 8.0–15.4, 12.1–14.6 and 12.1–14.3 for fractions I, II, III and IV, respectively. Values in the same range have been reported for catechin, epicatechin, gallocatechin and gallic acid (20.5, 10.2, 11.5, and 4.26 μmol TE/mg) (Yilmaz & Toledo, 2004) and for quercetin 3-glucoside, myricetin 3-arabinoside and *p*-coumaric acid (13.4, 14.4 and 8.6 μmol TE/mg) (Zheng & Wang, 2003). We have found values of 51.9, 47.7, 35.4, 9.6, 27.7 and 30.9 μmol TE/mg for the catechin, epicatechin, epigallocatechin, gallic acid, rutin and quercetin standards, respectively.

4. Conclusions

In conclusion, this is the first study focused on the identification and quantification of mashua non-anthocyanin phenolics. The compounds found in the different genotypes investigated were gallic acid, gallocatechin, epigallocatechin, procyanidin B_2 and epigallocatechin derivatives, different hydroxycinnamic and hydroxybenzoic acid derivatives and rutin and/or myricetin derivatives. Differences among genotypes were basically in the proportion of individual phenolics, as well as in the identity of minor peaks. The sequential use of liquid–liquid partition using ethyl acetate and of chromatography using Sephadex LH-20 was an efficient technique to fractionate mashua phenolics and to purify mashua proanthocyanidins. Proanthocyanidins significantly contributed to the total antioxidant activity of the mashua tubers but other phenolics such as phenolic acids, flavan 3-ols monomers, flavonols and anthocyanins are also contributors to the antioxidant capacity of this tuber. Proanthocyanidins of mashua tubers should thus be further studied.

Acknowledgements

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