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Original article

Postharvest storage and cooking techniques affect the stability of glucosinolates and myrosinase activity of Andean mashua tubers (*Tropaeolum tuberosum*)

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Summary The identification and quantification of Glucosinolates (Gls) via UPLC® MS-MS/PDA/qToF in ten mashua cultivars was carried out. Gls total contents were within the 4.9–54.2 μmol g^{-1} dry matter range, of which 96–99% corresponded to glucoaubrietin. Other less abundant Gls were glucotropaeolin and tentatively two isomers of hydroxybenzyl Gls. Postharvest refrigeration and shade storage conditions increased the content of Gls up to day 6 in 39.7% and 51.5% respectively. Sun exposure increased the Gls content in 40% up to day 3 but from day 6 considerable losses were attained (92% at day 15). Low correlation levels (R^2) between the Gls and myrosinase (MYR) activity of 0.57, 0.28 and 0.39 for the refrigeration, shade and sun exposure treatments were obtained. The cooking regimes tested, boiling, microwaving and baking totally inactivated MYR without affecting the Gls content.

Keywords Glucosinolates, mashua, myrosinase, postharvest storage and cooking, *Tropaeolum tuberosum*.

Introduction

Glucosinolates (Gls) are secondary metabolites present in plant families such as Brassicaceae, Capparaceae and Tropaeolaceae, among them, cabbage (white, purple), Brussels sprouts, cauliflower, broccoli, kale, mustard, turnip, radish and horseradish (Fahey *et al.*, 2001). Other sources of these compounds, less known are *Lepidium meyenii* (maca; Yábar *et al.*, 2011) and *Tropaeolum tuberosum* (mashua; Ramallo *et al.*, 2004). The structure of glucosinolates (*β*-thioglycoside *N*-hydroxysulfates) consists of a *β*-D-glucopyranosyl moiety linked via a sulphur atom to an *N*-hydroxyminosulfate ester, and of a modified amino acid side chain (Mohn *et al.*, 2007). Depending on the side chain, Gls can be classified as aliphatic, arylaliphatic and indol-3-ylmethyl derivatives. Gls can be hydrolysed by MYR (thioglucoside glucohydrolase, EC 3.2.3.147), an enzyme present in *Brassica* vegetables as well. To date, approximately 132 different Gls have been reported (Agerbirk & Olsen, 2012).

Glucosinolates are not biologically active. They only become active by the action of MYR. In vegetable cells, Gls are localised in the vacuoles of different cell types and are physically separated from MYR which is localised in the vacuole of specific cells named myrosin (Chen & Andreasson, 2001). When cell rupture occurs, Gls are rapidly hydrolysed by myrosinase, resulting in isothiocyanates, nitriles, ionic thiocyanate (SCN-) epithionitriles and oxazolidine-2-thiones (Wittstock & Halkier, 2002). The type of compounds formed depends on the nature of Gls, reaction conditions (e.g. pH, presence of ions) and on other compounds such as ascorbic acid and epithiospecifier proteins (EPS). Isothiocyanates (ITC) are considered as cancer preventive compounds given their ability to induce detoxifying phase II enzymes such as quinone reductase and glutathione-S-transferases (Tawfiq *et al.*, 1995).

Mashua is an Andean tuber rich in bioactive compounds such as polyphenols (Chirinos *et al.*, 2008) and Gls (Ramallo *et al.*, 2004). It belongs to the family of tropaeolaceas, and it is closely related to the ornamental garden nasturtium (*Tropaeolum majus*). Folk medicinal use of mashua claims, among others, health improvements related to kidney and liver pain, skin eczemas and prostate disorders (Grau *et al.*, 2000). *p*-methoxybenzyl glucosinolate (glucoaubrietin) has been identified as the predominant glucosinolate in mashua (Johns & Towers, 1981; Ramallo *et al.*, 2004; Martín & Higuera,

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2016). This glucosinolate is present in quantities relatively high when compared with other edible glucosinolate containing vegetables (Ramallo *et al*., 2004). Mashua tubers as other Andean tubers are subjected to different postharvest treatments (e.g. sun exposure, room temperature and refrigeration storage) and later cooking methods (e.g. boiling, microwaving and oven baking).

Different postharvest studies on this topic have been carried out in broccoli, Brussels sprouts, cauliflower, green, white and red cabbage, kale, pak choi and Chinese cabbage, radish and mustard. Storage of broccoli at room temperature or in refrigeration for 7 days provoked minor losses of Gls; similarly, steaming, microwave or stir-fry cooking did not provoke significant losses of Gls; however, boiling cooking resulted in significant losses of Gls due to their lixiviation in water (Song & Thornalley, 2007). The most important postharvest technologies to maintain the quality of broccoli are low temperature (<4 °C) and high relative humidity. Under these two conditions, the integrity of the cell is kept avoiding the action of MYR on Gls. Diverse studies indicate steaming as the most efficient method to maintain Gls intact in comparison to boiling cooking, microwaving, frying or vacuum processing (Lafarga *et al*., 2018). In addition, losses of Gls mainly occur due to lixiviation instead of the thermal effect on Gls.

The objective of the present study was to evaluate the influence of different postharvest storage treatments and cooking methods on the content and fate of mashua Gls.

Materials and methods

Plant material

Tubers of ten different cultivars of mashua were supplied by the International Potato Center (CIP, Lima, Peru). The tubers were grown at 3384 m above sea level, 11°46′45.89″ S latitude and 75°29′45.00″ W longitude (Region Junín, Peru). At harvest, three biological replicates for each cultivar composed of ~250 g each one were taken. Harvested tubers were immediately packed in paper bags and sent to the Universidad Nacional Agraria la Molina (Lima, Peru) where they were lyophilised and stored at −80 °C until the analysis were performed.

Chemicals

Sodium acetate, potassium dihydrogen phosphate and di-sodium hydrogen phosphate were purchased from Merck (Darmstadt, Germany), DEAE-Sephadex™ A-25 (GE Healthcare, Uppsala, Sweden), sulfatase from Helix pomatia type H-1 and sinigrin hydrate from horseradish were purchased from Sigma-Aldrich (USA). Benzyl glucosinolate was from Santa Cruz Biotechnology (Heidelberg, Germany). Solvents HPLC and LC-MS grade, formic acid from Sigma-Aldrich, methanol and acetonitrile, methanol and water LiChrosolv® were purchased from Merck (Darmstadt, Germany).

Identification and quantification of Gls in the different cultivars

Samples belonging to each of the ten cultivars were subjected to humidity analysis. In each of the ten cultivars, identification and quantification of Gls was carried out via UPLC® LC-MS qToF or UPLC® –PDA. MYR activity was also determined. The analytical methods are fully described below. The cultivar containing the highest amount of Gls was further selected to test different postharvest storage treatments and cooking methods.

Influence of postharvest storage treatments on mashua Gls content and MYR activity

The influence of three different postharvest storage treatments on the Gls content of the cultivar containing the highest Gls content previously selected were studied. These treatments corresponded to: refrigeration, shade and sun exposure. The shade and sun-exposed treatments were carried out in the same place where the tubers were cultivated. The "shade" treatment was undertaken in a room where the tubers were extended on polypropylene bags on top of the floor and covered during the night with "yute" bags to protect the tubers from frosts. For the "sun-exposed" treatment, mashua tubers were extended on polypropylene bags outside during 9 h day^{-1} and during the night the tubers were covered with "yute" bags to protect them against frosts (−2 to 8 °C). In addition, daily between 13 and 14 h, tubers were flipped in order to obtain a homogenous exposure. Similar sun-exposure practices are carried out by the peasant communities. The "refrigeration" treatment was performed in a domestic refrigerator at ~4 °C. Tubers were placed in perforated paper bags. For all storage conditions, samples were taken at 0, 3, 6, 9, 12 and 15 days. Tubers were immediately packed in paper bags (~500 g) and sent to Universidad Agraria La Molina (Lima, Peru) where they were lyophilised and stored at −80 °C. The three samples taken for each time were worked and analysed independently.

Influence of cooking processes on mashua Gls content and MYR activity

Three cooking methods were applied: boiling, oven baking and microwaving. Three replicates were

considered for each cooking method. For the boiling method, ~500 g of whole unpeeled mashua tubers were added to 1.5 L of distilled water and then boiled at 96 °C (±2) for 15 min. Cooking in a Samsung microwave oven was done by placing unpeeled mashua tubers in Ziploc bags (~500 g) with 100 mL of distilled water at 1000 W for 5 min. Also the unpeeled mashua tubers (~500 g) were placed in a preheated oven at 180 °C and were processed for 25 min. After each cooking time, samples were cooled rapidly and stored at −20 °C in plastic bags for further analysis. Glucosinolates were determined and dry matter after cooking processes was considered for quantification.

Analytical determinations

Extraction and desulfation of glucosinolates

The protocol described by Verkerk *et al*. (2001) and Yábar *et al*. (2011) with some modifications was used. Freeze dried mashua (~0.2 g DM) was weighed and mixed with 10 mL of 70% hot methanol. The samples were incubated in a bath at 75 °C for 25 min and then centrifuged at 4500 ***g*** for 20 min. The supernatant was collected and the cake re-extracted with 10 mL of 70% hot methanol, as for the first extraction. Both supernatants were combined and mixed. A part of the extract was used for the identification of the intact Gls. An aliquot was desulfated on 0.5 × 10 cm glass column containing 100 mg of DEAE-Sephadex A-25. Each column was washed with three volumes of milli-Q water. Then, 1 mL of extract was added to each column, which was later washed twice with the same amount of water in order to remove non-retained soluble compounds. Sulfatase (100 μL), purified according to Crocoll *et al*. (2016), was added to the column. Incubation was carried out overnight at room temperature. Desulfoglucosinolates were eluted with 2 mL of milli-Q water. The eluate was filtered through a 0.22 μm filter (13 mm, Millipore Corporation, Billerica, MA, USA) and this sample was suitable for HPLC analysis.

Identification and quantification of glucosinolates

Identification of intact (non desulfated) Gls was performed on an ultra-performance liquid chromatography (ACQUITY UPLC I Class, Waters Corp., Milford, MA, USA) equipped with an Acquity BEH column (1.8 μm, 50 × 2.1 mm, Waters Corp) connected to a quadrupole time of flight mass spectrometer (Xevo G2-XS QTof, Waters Corp). The mobile solutions were water with 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B). Intact or desulfoglucosinolates were analysed, the injection volume was 1 μL. The total running time was 21 min, with a gradient as follows: 2% B for 2 min, then in 12 min to 17% B, then in 1 min to 100% B, for 3 min 100% B and re-equilibrated for 3 min with 98% A. The column was kept at 30 °C and the flow rate was 0.2 mL min^{-1}. Mass spectra were acquired using electrospray ionisation negative mode (−3.5 kv) over the range of m/z 50–1200. The desolvation temperature was 300 °C, the cone gas flow was 20 L h^{-1}, and the desolvation gas flow was 600 L h^{-1}. MS/MS fragmentation process was accomplished at normalised collision energy ramped from 15 to 50 eV. The accurate mass and composition of the precursor and fragment ions were calculated and sequenced using the MassLynx software (Waters Corp., Milford, MA, USA). Monoisotopic mass of the molecular ion $[M\text{-}H]^-$ with a mass error <5 ppm and fragmentation patterns were used for identification by comparison with literature references and online databases (PubChem). A standard of desulfoglucotropaeolin was analysed under the same conditions.

The quantification of desulfoglucosinolates was performed by ACQUITY UPLC® H Class equipped with PDA eλ Detector (Waters Corp., Milford, MA, USA). The column and all the conditions of analysis were the same as those previously used for identification. Detection was carried out at 229 nm. Molar concentrations of individual glucosinolates and relative response factors (Brown *et al*., 2003; Ramallo *et al*., 2004) were used to correct for absorbance differences between the standard (prop-2-enyl glucosinolate, sinigrin) and the other components of the extract. Total Gls were expressed as μmol g^{-1} DM and determined by adding up all individual Gls. The standard curve was built with desulfosinigrin.

Myrosinase activity (thioglucoside glucohydrolase, EC 3.2.3.1)

The methodology described by Li & Kushad (2004) with some modifications was used. Briefly, to 33.3 mM pH 6.5 phosphate buffer, 0.5 mM EDTA and 0.25 mM ascorbic acid (buffer A) were added, the buffer was cooled down to 4 °C. Five millilitres of buffer A with 0.25 g of freeze dried mashua were mixed. Extraction was carried out at 4 °C for 60 min. The enzymatic extract was obtained after centrifugation at 3500 ***g*** and 4 °C for 20 min. One aliquot of the extract was dialysed (Dialysis tubing cellulose, D9652-100FT, Sigma-Aldrich, St. Louis, USA) at 4 °C against 100X volume buffer A for 16 h. The supernatant was collected and used as crude extract for myrosinase determination. The reaction mixture consisted of 900 μL of extract and 100 μL of 0.2 mM sinigrin which were placed in a quartz cuvette with 1 cm path length. The cuvette was placed in a UV-vis spectrophotometer microplate (Bio-Tek Instruments Inc, model EONC, Winooski, Vermont USA) equipped with a temperature compensated chamber set at 37 °C and a stirring system. The reaction was carried out at 37 °C, mixing 900 μL of

0.12 mM of sinigrin dissolved in buffer A and 100 μL of the dialysed extract. Decline in optical density as a result of sinigrin breakdown was plotted at 227 nm and at 37 °C over a 25-min period. The rate of absorbance decline within the linear phase of the graph was used to calculate the enzyme activity. One unit of activity was defined as the amount of myrosinase that catalyses the hydrolysis of 1 μmol sinigrin per minute, under the conditions described above. Unit activity was calculated according to the following formula:

$$U = \Delta A . \frac{1}{\text{e.l}} . \frac{V_A}{V_E} . 10^3$$

where the extinction coefficient (e) of sinigrin at 227 nm is 6784 M^{-1} cm^{-1}, the cuvette path length (l) is 1 cm, total volume of the assay mixture (V_A) is 1.0 cm^3 and the volume of enzyme solution (V_E) is 0.1 cm^3.

Ascorbic acid content

The method reported by Klimczak & Gliszczyńska-Świgło (2015) with some modifications was used. To 0.3 g of freeze dried sample, 10 mL of a solution containing 3% metaphosphoric acid and 8% acetic acid were added and homogenised for 10 min. The mixture was centrifuged at 6500 ***g*** and 4 °C for 25 min. The extract was analysed using an ultra-performance liquid chromatography (ACQUITY UPLC I Class, Waters Corp., Milford, MA, USA) equipped with an Acquity BEH column (1.8 μm, 50 × 2.1 mm, Waters Corp.), gradient of mobile phase composed of methanol (solvent A) and 5 mmol L^{-1} KH_2PO_4, pH 2.65 (solvent B) was used according to the following gradient: 2% A during 1 min, in 3 min to 65% A, in 0.5 min to 95% A, during 2 min 0.5% A, in 0.5 min to 2% A and during 3 min 2% A. Results were expressed in mg L-ascorbic acid per g DM.

Statistical analysis

The results were reported as mean ± standard deviation (SD) of three independent replicates. One-way analysis of variance (ANOVA) was used to compare the means, and Tukey's test was used to assess statistical significant differences among treatments ($P < 0.05$). All statistical analyses were performed with Statgraphics® Centurion XV (Stat Point Technologies, Inc., Warrenton, VA, USA).

Results and discussion

Glucosinolates and myrosinase activity content in different cultivars of mashua

Four molecules with *m*/*z* 424.0365 and 424.0416 (hydroxybenzyl glucosinolate, two possible isomers), 408.0465 (glucotropaeolin) and 438.0548 (glucoaubrietin) were detected in the extracted chromatograms for the Gls (non desulfonated) from mashua and obtained via ESI-MSe (negative) analysis (Figure S1). To confirm the identity of the Gls, desulfonated samples were analysed via ESI-MS/MS (negative) (Fig. 1). The most abundant compound corresponded to 4-methoxybenzylglucosinolate (glucoaubrietin) given that for the desulfoglucoaubrietin: 2MDS-H = 717.1946 (dimer of molecular ion), MDS-H = 358.0937 (most abundant fragmented molecular ion) and MDS-H-162 = 196.0317 (deglucosylated desulfoglucoaubrietin molecular ion) were obtained. These values correspond to the ones reported by Kusznierewicz *et al.* (2008). For benzylglucosinolate (glucotropaeolin): 2MDS-H = 657.1716 (dimer of molecular ion), MDS-H = 328.0835 (molecular ion, most abundant fragment) and MDS-H-162 = 195.0315 were observed. The possible isomers *p*-hydroxybenzyl glucosinolate (glucosinalbin) and *m*-hydroxybenzyl glucosinolate (glucolepigramin): 2MDS-H = 689.1588 and 2MDS-H = 689.1636 (dimers of molecular ion), MDS-H = 344.0763 and MDS-H = 344.0791 (molecular ion, most abundant fragment) were tentatively identified. Ortega *et al.* (2006) also found hydroxybenzyl glucosinolate in *T. tuberosum*, and Li *et al.* (2001) reported the presence of *p*- and *m*-hydroxybenzyl glucosinolate in *Lepidium meyenii* (maca). The analysis of fresh maca (results not shown) allowed us to confirm the presence of both isomers. Similar profiles of Gls were found for all the analysed cultivars of mashua. In addition, Ramallo *et al.* (2004) and recently Martín & Higuera (2016) found that the main Gls in mashua was glucoaubrietin, and Martín & Higuera (2016) also pointed out the possible presence of *p*-hydroxybenzyl glucosinolate.

The total content of Gls and myrosinase activity for all mashua cultivars is displayed in Table 1. Gls of the different cultivars were within the 4.9–54.2 μmol g^{-1} DM (96–99% corresponded to glucoaubrietin) range and are in agreement with the values reported by Ortega *et al.* (2006) (0.27–50.74 μmol g^{-1} DM), Ramallo *et al.* (2004) (36.5–90.0 μmol g^{-1} DM) and higher than the range values reported by Martín & Higuera (2016) (0.3–25.8 μmol g^{-1} DM). The purple cultivars ARB 5241 and DP 0224 (Figure S2) displayed the highest concentration of Gls with values of 54.2 and 51.2 μmol g^{-1} DM respectively. These purple cultivars also contained higher values of Gls compared to other vegetables such as green cauliflower, purple cauliflower and rutabaga (5.26, 8.26 and 7.34 μmol g^{-1} DM, respectively; Kapusta-Duch *et al.*, 2016); broccoli-buds and broccoli-stalks (21.4 and 13.6 μmol g^{-1} DM respectively; Rybarczyk-Plonska *et al.*, 2016); maca (31.4–36.2 μmol g^{-1} DM; Yábar *et al.*, 2011); and pak choi (10.9 μmol g^{-1} DM; Yang *et al.*, 2010).

For MYR activity, a range of 2.9–3.8 U g^{-1} DM was found with non-significant differences ($P > 0.05$)

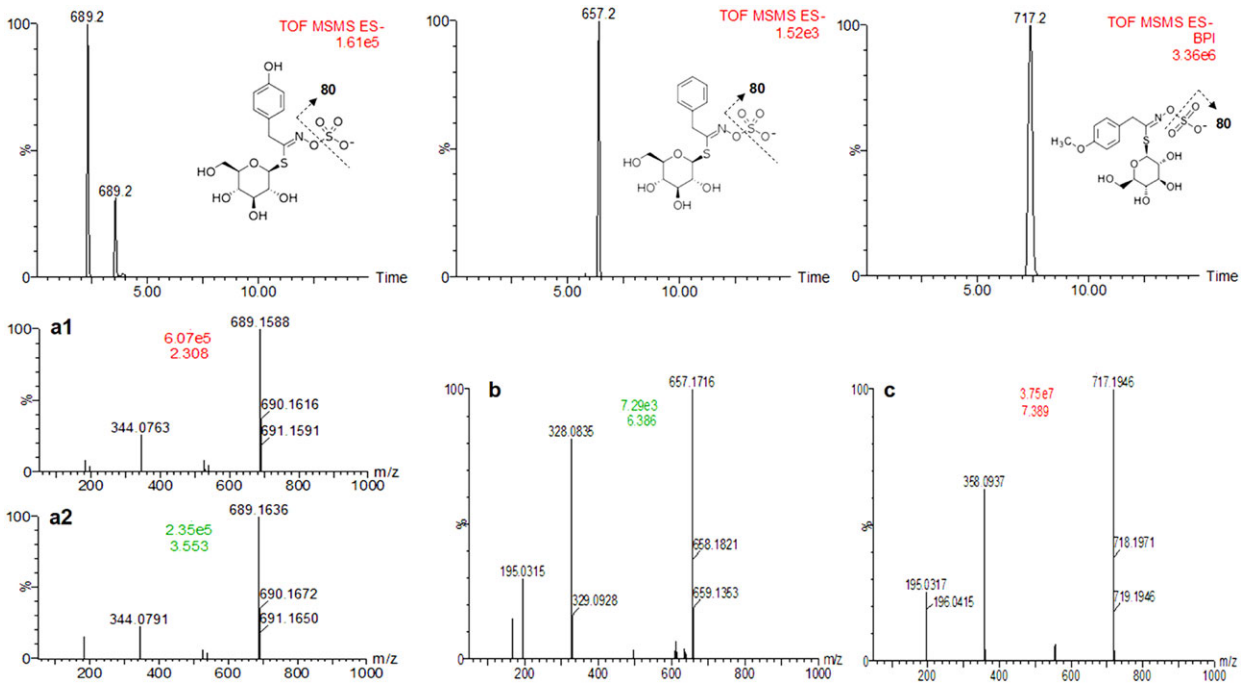

Figure 1 UPLC-qTOF-MS-MS base peak ion (BPI) chromatogram extracted from the desulfoglucosinolate extract from mashua ARB 5241 desulfated and MS–MS spectra obtained, (a1, a2) *m*/*z* 344.0791 and 344.0763 for *p*- and *m*-4-hydroxybenzyl- desulfoglucosinolate (tentative) (b) *m*/*z* 328.0835 benzyl desulfoglucosinolate and (c) *m*/*z* 358.0937 4-metoxybenzyl desulfoglucosinolate.

Table 1 Total glucosinolate content and myrosinase activity for the different analysed mashua cultivars

Cultivar	Glucosinolate (μmol g^{-1} DM)	Myrosinase (U g^{-1} DM)
20 Isaño	19.9 ± 2.5^{d}	3.6 ± 0.3^{a,b}
03 Chiara	48.0 ± 0.4^{a}	2.9 ± 0.2^{b}
19 K'ello	12.9 ± 0.8^{d}	3.1 ± 0.2^{a,b}
15 Zapallo	29.4 ± 1.0^{c}	3.2 ± 0.2^{a,b}
02 Isaño	32.4 ± 1.4^{b,c}	3.5 ± 0.4^{a,b}
01 K'ello	38.7 ± 5.9^{b}	3.7 ± 0.5^{a,b}
ARV 5366	4.9 ± 0.4^{e}	3.4 ± 0.2^{a,b}
ARB 5576	20.4 ± 0.4^{d}	3.8 ± 0.2^{a}
ARB 5241	54.2 ± 4.9^{a}	3.3 ± 0.3^{a,b}
DP 0224	51.2 ± 4.3^{a}	3.0 ± 0.2^{a,b}

Results are mean ± SD (n = 3). Columns with the same superscript small letter are not significantly different ($P > 0.05$).

for the different cultivars. No correlation between MYR activity and content of Gls was found.

Influence of storage type on glucosinolate content and myrosinase activity

Results concerning the evolution of the content of Gls subjected to three different postharvest treatments: refrigeration, shade and sun exposure are displayed in Table 2. An increase in the content of Gls up to day 12 of refrigeration and 9 days under shade were observed. For the sun-exposed treatment, a significant increase ($P < 0.05$) of Gls up to day 3 was evident. Maximum concentrations of Gls were obtained under refrigeration, shade and sun exposure at 12, 6 and 3 days respectively (77.01 (Δ 44.8%), 80.77 (Δ 51.9%), 74.86 (Δ 40.8%) μmol g^{-1} DM, respectively). Non-significant differences ($P > 0.05$) were observed in the concentration of Gls at the maximum concentration attained with the different treatments. After 12 days of refrigeration and 9 days of shade, a significant decrease ($P < 0.05$) in Gls was observed reaching values similar to the initial content at day 15. The trend of total Gls is similar to the trend obtained for glucoaubrietin. Similar results were observed by Yang *et al.* (2010) for pak choi leaves (*Brassica rapa* L. ssp. *chinensis* var. *communis*) stored at 4 °C; total Gls increased up to days 5 and 7 and then decreased to its initial value at day 9. However, the behaviour of mashua tubers during storage was different to the observed with broccoli by Rodrigues & Rosa (1999). These authors did not find any change at 4 °C, but an important decrease at 20 °C; and Toivonen & Forney (2004) observed decreases of Gls of 31% and 82% in stored broccoli at 4 and 20 °C respectively. Song &

Table 2 Content of glucosinolates and myrosinase activity in mashua tubers subjected to refrigeration, shade or sun exposure for 15 days

Treatment/time (day)	Hydroxybenzyl glucosinoate* (μmol g^{-1} DM)	Hydroxybenzyl glucosinolate* (μmol g^{-1} DM)	Benzyl glucosinolate (μmol g^{-1} DM)	4-Methoxybenzyl glucosinolate (μmol g^{-1} DM)	Total glucosinolates (μmol g^{-1} DM)	Myrosinase (U g^{-1} DM)
Refrigeration						
0	0.12 ± 0.00^{ab}	0.09 ± 0.02^{ab}	0.17 ± 0.00^{cd}	52.80 ± 0.98^{cd}	53.18 ± 0.82^{cd}	3.1 ± 0.5^{ef}
3	0.14 ± 0.02^{ab}	0.12 ± 0.00^{a}	0.19 ± 0.00^{a}	56.01 ± 0.28^{cd}	56.46 ± 0.27^{cd}	3.5 ± 0.1^{de}
6	0.16 ± 0.03^{ab}	0.11 ± 0.00^{a}	0.18 ± 0.01^{abc}	74.12 ± 4.68^{a}	74.56 ± 4.77^{a}	4.7 ± 0.1^{bc}
9	0.16 ± 0.00^{ab}	0.11 ± 0.00^{a}	0.15 ± 0.00^{ef}	56.24 ± 1.72^{cd}	56.66 ± 1.76^{cd}	5.1 ± 0.0^{b}
12	0.14 ± 0.00^{ab}	0.13 ± 0.00^{a}	0.18 ± 0.00^{ab}	76.56 ± 0.33^{a}	77.01 ± 0.35^{a}	6.8 ± 0.3^{a}
15	0.11 ± 0.01^{abc}	0.09 ± 0.00^{ab}	0.15 ± 0.00^{f}	48.16 ± 6.42^{d}	48.51 ± 6.60^{d}	3.9 ± 0.1^{d}
Shade						
0	0.12 ± 0.00^{ab}	0.09 ± 0.02^{ab}	0.17 ± 0.00^{bcd}	52.80 ± 0.98cd	53.18 ± 0.82^{cd}	3.1 ± 0.5^{ef}
3	0.20 ± 0.03^{a}	0.13 ± 0.00^{a}	0.18 ± 0.00^{ab}	76.95 ± 1.87^{a}	77.46 ± 1.94^{a}	2.4 ± 0.2^{fgh}
6	0.18 ± 0.03^{a}	0.13 ± 0.00^{a}	0.17 ± 0.01^{cde}	80.29 ± 0.47^{a}	80.77 ± 0.50^{a}	5.4 ± 0.3^{b}
9	0.15 ± 0.00^{ab}	0.13 ± 0.00^{a}	0.17 ± 0.00^{bcd}	73.22 ± 3.06^{ab}	73.67 ± 3.08^{ab}	3.9 ± 0.1^{d}
12	0.14 ± 0.01^{ab}	0.11 ± 0.01^{a}	0.17 ± 0.00^{bcd}	62.63 ± 630^{bc}	63.05 ± 6.37^{bc}	3.4 ± 0.4^{de}
15	0.13 ± 0.00^{ab}	0.11 ± 0.00^{a}	0.16 ± 0.00^{def}	57.58 ± 0.43^{cd}	57.98 ± 0.40^{cd}	2.5 ± 0.4^{fgh}
Sun exposure						
0	0.12 ± 0.00^{ab}	0.09 ± 0.02^{ab}	0.17 ± 0.00^{bcd}	52.80 ± 0.98^{cd}	53.18 ± 0.82^{cd}	3.1 ± 0.5^{ef}
3	0.16 ± 0.00^{ab}	0.13 ± 0.01^{a}	0.18 ± 0.00^{ab}	74.39 ± 2.97^{a}	74.86 ± 3.02^{a}	4.0 ± 0.1^{cd}
6	0.09 ± 0.00^{ab}	0.07 ± 0.00^{b}	ND	23.12 ± 0.09^{e}	23.28 ± 0.07^{e}	4.0 ± 0.2^{cd}
9	ND	ND	ND	7.23 ± 0.30^{f}	7.23 ± 0.38^{f}	3.2 ± 0.2^{ef}
12	ND	ND	ND	5.53 ± 0.01^{f}	5.53 ± 0.08^{f}	2.9 ± 0.1^{efg}
15	ND	ND	ND	3.75 ± 0.25^{f}	3.75 ± 0.29^{f}	1.9 ± 0.1^{h}

*Possible isomers *m*- and *p*-hydroxybenzyl glucosinolate. Quantified as glucosinalbin.
ND, not detected. Results are mean ± SD ($n = 3$). Columns with the same superscript small letter are not significantly different ($P > 0.05$).

Thornalley (2007) reported that room storage conditions caused no significant or minimal losses of Gls contents in broccoli, cauliflower and green cabbage. Rybarczyk-Plonska *et al.* (2016) reported that Gls contents were not changed in broccoli flower buds during pre-storage at low temperatures (0 or 4 °C) for 4 or 7 days, even suggesting that the combination of pre-storage at 0 °C followed by storage at 10 °C, maximises the level of Gls in broccoli florets. Paulsen *et al.* (2018) reported for broccoli florets stored at 4 °C that the total Gls are maintained but significantly decrease when stored at 15 °C. These controversial results demonstrate the necessity to carry out independent studies for each species under well-defined postharvest conditions. Gls content increase in mashua during the early stages of storage is closely linked to stress factors such is temperature. Recently, Villarreal-García *et al.* (2016) pointed out that stress causing factors (e.g. storage temperature) trigger the activation of the secondary metabolism of broccoli increasing it Gls content. However, the mechanisms related to the synthesis of Gls in response to stress are still unknown (Rybarczyk-Plonska *et al.*, 2016). Biotic and abiotic factors which influence secondary metabolite production have different applications, such as improvements in the production of phytochemicals (Ramakrishna & Ravishankar, 2011).

The behaviour during sun exposure was very different compared to the previously described treatments. A considerable increase of Gls was observed at 3 days and then a decrease, with lower values from day 9 (Table 2) and losses greater than 85%. Yábar *et al.* (2011) reported sun-exposure postharvest drying with three different maca ecotypes using similar conditions (altitude and climate) to the conditions used in this work. A different behaviour was observed; Gls increased during 15 and 30 days and an important decrease was observed at day 45 with total Gls losses of 37.6%, 20.4% and 52.4% for the yellow, red and black maca ecotypes, respectively. These losses according to Yábar *et al.* (2011) were due to the action of MYR activity, which acts after cellular rupture triggered by freezing and thawing due to the temperature conditions from −3 to 20 °C in the storage location. Water losses due to evaporation could cause cell rupture and thus promote the liberation of Gls and MYR. Under these environmental conditions, the faster and greater degradation of Gls observed in mashua tubers could be related to a higher MYR activity and/or to a higher content of ascorbic acid. However, it is not possible to compare the results of MYR activity with those reported by Yábar *et al.* (2011), since different methods of analysis were used. The high Gls losses between 3 and 6 days of sun exposure (Table 2) is

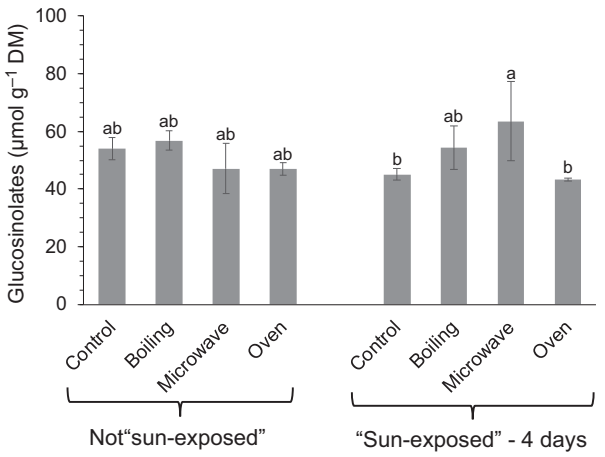

Figure 2 Glucosinolate content in mashua "non-sun exposed" and "sun-exposed" and further subjected to different cooking regimes. Controls correspond to fresh mashua and 4-day sun-exposed mashua tubers.

correlated with the high MYR activity and important content of vitamin C (Figure S3) since ascorbic acid is an important cofactor for the activity of this enzyme. The lower rate of reduction of Gls from day 9 is related to the lower amount of substrate available and to the reduction of the humidity that leads to a lower rate of enzymatic activity. It was also observed that the lowest MYR activity was obtained for the sun exposure treatment, compared to refrigeration and shade storage. Thus, it is feasible that inhibition of enzyme occurred due to accumulation of transformation products of Gls formed in higher quantities in the sun exposure treatment. Lim *et al.* (2015) reported for radish roots that the isothiocyanates formed from Gls inhibited the myrosinase enzyme. The linear correlation coefficients (R^2) between total Gls and MYR activity for the refrigeration, shade and sun-exposure treatments corresponded to 0.57, 0.28 and 0.39 respectively. In addition, Lim *et al.* (2015) previously reported poor correlations between the Gls content and myrosinase activity in twenty-seven accessions of horseradish roots.

Effect of cooking regime on total glucosinolates and myrosinase activity

Thermal treatment of vegetables that contain Gls could cause losses through different mechanisms such as enzymatic transformation, thermal degradation and lixiviation to the heating medium. The effect of the different cooking processes on Gls content is presented in Fig. 2, after boiling, microwaving and oven cooking. MYR activity was also determined after each cooking regime, being MYR completely inactivated after the application of each cooking regime. Up to our knowledge, no reports regarding the thermal stability of MYR enzyme in mashua has been reported. However, reports on other species are available. Myrosinase extracted from cabbage had an optimal temperature of 60 °C and their activity was destroyed after heating at 70 °C for 30 min (Yen & Wei, 1993); the myrosinase purified from horseradish exhibited high activity at broad pH (pH 5.0–8.0) and temperature (37 and 45 °C; Li & Kushad, 2004). Inactivation of mashua MYR does not mean that the beneficial compounds (ITCs, nitriles, etc.) will not be formed. Gls will reach the colon and will be transformed by the bacterial enzymes present (Mithen *et al.*, 2000). However, different authors coincide that this conversion is less efficient than by the action of the enzyme naturally present in the vegetable (Holst & Williamson, 2004).

Non-significant differences ($P > 0.05$) were found for the content of total Gls for all cooking regimes except for 4-day sun-exposed (control) and 4-day sun-exposed (baking) treatments (Fig. 2) with lower values ($P < 0.05$). Contrary, sun-exposed samples subjected to boiling or microwaving displayed a similar content of Gls compared to non-treated mashua (control). These treatments soften tissues and facilitate the extraction and quantification of all Gls. In addition, the microwaving cooking regimes have been previously used to inactivate myrosinase. Kapusta-Duch *et al.* (2016) reported Gls losses after a boiling process of 6.6% in rutabaga (*Brassica napus*) and in average 69% in green and purple cauliflower, being more sensitive to the process, the indolic Gls. The thermostability of Gls is dependent on the structure of the lateral chain (Hanschen *et al.*, 2014). The structure of the lateral chain of the mashua Gls is of aromatic nature (*p*-methoxybenzylglucosinolate, >96%) displaying higher thermal stability. Another important factor to consider is the structure of the solid matrix (Dekker *et al.*, 2009) which is related to the lixiviation in boiling water. Whole tubers (with peel) would not display lixiviation of Gls. Up to our knowledge, there are no reports related to losses of Gls in tubers. Most of the studies have been realised in brassicaeas. Among nine publications related to boiling of Gls, three reported losses of <50%, five reported losses >50% and only one did not report any loss (Palermo *et al.*, 2014).

Conclusions

A great variability of Gls content was found among the ten studied mashua cultivars. The purple mashua cultivars displayed the highest Gls contents. These purple cultivars are richer in Gls than other species of the family Brassicaceae. The major Gls corresponded to glucoaubrietin (>96%) in all mashua cultivars.

Postharvest storage treatments such as shade and/or refrigeration up to 15 days did not affect the content of Gls, but sun exposure for 15 days resulted in more than 86% Gls losses. Cooking regimes such as boiling, microwaving and baking completely inactivated myrosinase activity and did not have any influence in the content of Gls. The transformation of Gls into bioactive compounds would take place by the intestinal microflora. Further studies in our research group are being performed regarding the influence of the produced bifidobacteria and lactic acid bacteria on the transformation of Gls.

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Conflict of interest

The authors declare no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. UPLC-qTOF-MSe BPI chromatograms extracted from the glucosinolate extract from mashua ARB 5241 and MS spectra obtained from (a1, a2) m/z 424.037, 4240416 for *p*- and *m*-hydroxybenzyl glucosinolate (tentative) (b) m/z 408.042 glucotropaeolin (c) m/z 438.053 glucoaubrietin.

Figure S2. Coloured pictures of the different mashua cultivars.

Figure S3. Myrosinase activity and content of ascorbic acid in mashua subjected to different postharvest storage times.