Food Chemistry 127 (2011) 1576–1583

Contents lists available at ScienceDirect

Food Chemistry

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

Glucosinolate content and myrosinase activity evolution in three maca (*Lepidium meyenii* Walp.) ecotypes during preharvest, harvest and postharvest drying

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ARTICLE INFO

Article history:
Received 14 November 2010
Received in revised form 24 December 2010
Accepted 4 February 2011
Available online 26 February 2011

Keywords:
Maca
Lepidium meyenii
Pre and post-harvest
Glucosinolates and myrosinase activity

ABSTRACT

Glucosinolate profiles, glucosinolate contents and myrosinase activity were evaluated in yellow, red and black hypocotyls of maca during pre-harvest, at harvest and during post-harvest drying. At harvest, six glucosinolates (GLs) were identified: 5-methylsulfinylpentyl, 4-hydroxybenzyl, benzyl, 3-methoxybenzyl, 4-hydroxy-3-indolylmethyl and 4-methoxy-3-indolylmethyl, of which benzyl glucosinolate was the most abundant in the three ecotypes, representing 80% of the total GLs. A significant increase in GLs was observed for the three ecotypes during the 90 days before harvest and during the 15–30 days of post-harvest drying. This was followed by an important decrease of GLs during the 30–45 day period, which was attributed to cell breakdown, due to fluctuations in temperatures during the drying process, and was correlated with a high myrosinase action. During the last period of post-harvest drying, GLs were much lower and correlated to lower myrosinase activity and lower maca hypocotyl humidity. A combination of artisanal and other processing techniques should be utilised, in order to best preserve maca glucosinolates.

1. Introduction

Maca (*Lepidium meyenii* Walp.) is a traditional Andean crop belonging to the Brassicaceae family that grows at altitudes between 3500 and 4500 m above sea level in the central Andean region of Peru. This area is characterised by barren and rocky terrains with intense sunlight, strong winds and freezing temperatures. Few crops belonging to the Solanaceae family can survive in such harsh conditions (Flores, Walker, Guimarães, Bsid, & Vivanco, 2003).

The nutritional value of this crop has been largely studied (Dini, Migliuolo, Rastrelli, Saturnino, & Schettino, 1994; Valentová et al., 2006) and stimulant effects have been observed. In the Peruvian Andes, folklore use of this crop includes treatment of infertility, increase of mental and physical energy and treatment of menopause. Maca meal nutritional properties and apparent effects on reproductive and sexual performance in rats and humans have been extensively documented (Gonzales, Ruiz, Gonzales, Villegas, & Córdova, 2001; Gonzales, Córdova, et al., 2001; Gonzales et al., 2004). However, studies related to the improvement of sexual performance are still controversial (Wang, Wang, McNeil, & Harvey, 2007). Maca, in addition, showed anti-proliferative functions and slowed down the prostate weight increase induced by testosterone treatment (Gonzales et al., 2005, 2006). Rats that were orally administered red maca showed beneficial effects in the treatment of prostatic hyperplasia (BPH) experimentally induced by testosterone (Gasco, Villegas, Yucra, Rubio, & Gonzales, 2007). Proposed mechanisms for this anti-proliferative function included its ability to scavenge free radicals and cytoprotection under oxidative stress conditions (Lee, Dabrowski, Sandoval, & Miller, 2005; Sandoval et al., 2002). The presence of alkaloids and sterols in maca may also have contributed to its proposed anti-cancer activity (Wang et al., 2007) as well as its glucosinolates and derivatives (Fahey, Zalcmann, & Talalay, 2001). The presence of phytosterols and other secondary metabolites in maca are related to its anti-postmenopausal osteoporosis function (Wang et al., 2007).

Glucosinolates (GLs) are the most important secondary metabolites in maca (Jones, 1981) being most of them of the aromatic type (Dini, Terone, & Dini, 2002; Flores et al., 2003; Li, Ammermann, & Quirós, 2001), of which glucotropaeolin is the most abundant. According to Clément et al. (2009) 80–90% of the total GLs in maca corresponded to glucotropaeolin. Maca GLs are present in different organs of the plant (e.g., seeds, sprouts). GLs in maca vary in content and type depending on plant age and hypocotyl colour (Clément et al., 2010). Glucosinolate content in fresh maca is about 1%, which is about 100 times more than that found in cruciferous crops, such as cabbage, cauliflower and broccoli (Li et al., 2001).

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doi:10.1016/j.foodchem.2011.02.021

The highest total glucosinolate content in maca is found in fresh hypocotyls, followed by seeds, sprouts, dried hypocotyls and fresh leaves (Li et al., 2001). Myrosinase is involved in the breakdown of GLs into isothiocyanate, thiocyanate and nitriles (Fahey et al., 2001). In intact cells, GLs and myrosinase are in different compartments. Thus, cell damage favours GL breakdown by myrosinase. The effects of variety, cultivation practices, harvest time, climate and processing on GL content have been extensively documented (Kushad et al., 1999; Mrkic, Redovnikovic, Jolic, Delonga, & Dragovic-Uzelac, 2009; Rosa, Heaney, Portas, & Roger, 1996; Verkerk, Dekker, & Jongen, 2001). However, to our knowledge, there are no studies related to the GL content and fate of maca during post-harvest drying.

The functional and nutraceutical potential of maca has resulted in a significant increase in its production, resulting from a sustainable increase in exports (from 189 to 602 Tm between 1999 and 2009). The main export products are: maca flour (dried and milled), gelatinised maca flour (dried, extruded and milled), plain maca or encapsulated and hydroalcoholic extracts. The main export destinations are USA and Japan (Aliaga & Espinoza, 2007).

Regardless of the maca product, post-harvest drying of maca is carried out in an artisanal way in the places of production, before other types of processing (extrusion, milling, etc.) are applied. Briefly, environmental drying is carried out for approximately 90 days in extreme temperature conditions ranging from −10 to 15 °C. These extreme environmental conditions together with the handling procedures during harvest and post-harvest can have a significant effect on the final glucosinolate content in maca.

Thus, the main objective of this work was to understand the effect of the series of events happening during pre-harvest, harvest and post-harvest drying of three maca ecotypes on glucosinolate profile, content and myrosinase activity. Understanding the events of GL breakdown in maca during pre-harvest, harvest and post-harvest drying would suggest better alternatives to reduce GL losses and result in maca products with a higher added value.

2. Materials and methods

2.1. Plant material

Three different maca ecotypes (yellow, red and black) were selected from a commercial plantation. Cultivation was carried out in November in the community of San Pedro de Cajas at 4200 m above sea level (Junín, Perú). Maca tubers were harvested in July. Random samples were taken as follows: (a) samples from 10 plants were taken (∼2 kg) 90, 45, 30 and 15 days before harvest time, (b) at harvest and (c) after 15, 30, 45, 60, 75 and 90 days of post-harvest drying. During post-harvest handling, hypocotyls were air conditioned for 2 days, then placed in 50-kg net sacks and left in such conditions for 3 days. After this period, they were transported to the drying facility where they were spread in beds of 10 cm height. During the night, maca tubers were covered with tents to protect them from freezing conditions (−10 °C). Drying temperatures ranged from −10 to 15 °C and relative humidity from 70% to 95%. Maca tuber samples were transported to the laboratory, where they were cleaned with tap and distilled water, dried with paper towel and packaged in black polythene bags and stored at −20 °C until analysis.

2.2. Chemicals

Methanol was from Fermont (México, DF); HPLC-grade acetonitrile, sodium acetate, sodium dihydrogen phosphate monohydrate, di-sodium hydrogen phosphate dihydrate from Merck (Darmstadt, Germany) were used. DEAE Sephadex A-25, sulfatase from *Helix pomatia* type H-1 and sinigrin hydrate (Biochemika) were purchased from Sigma–Aldrich. Benzylglucosinolate was from KVL (Copenhagen, Denmark) and rapeseed BC-367 ERM from IRMM (Geel, Belgium) were used as glucosinolate standards.

2.3. Moisture

Moisture was determined according to the AOAC (1998) method.

2.4. Extraction of glucosinolates

The protocol described by Verkerk et al. (2001) with some modifications was used. Maca hypocotyls were frozen in liquid nitrogen and immediately cut and crushed to avoid myrosinase action. Maca pulp (∼1 g DW) 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. The extracted GLs were purified on a 1.5 cm DEAE Sephadex A-25 anion exchange column. The column was washed twice with 1 ml of Millipore water, and 2 ml of extracted GLs were loaded and washed twice with 1 ml of 20 mM NaAc solution. Sulphatase (100 μl of a 25 mg/ml solution) was added to the column. Incubation was carried out overnight at room temperature. Desulfoglucosinolates were eluted with 4 ml of Millipore water. The eluate was filtered through a 0.22-μm filter (13 mm, Millipore Corporation, Billerica, MA) and this sample was suitable for HPLC analysis.

2.5. HPLC analysis of glucosinolates

Maca desulfoglucosinolates were analysed using high-performance liquid chromatography. An HPLC Waters 2695 Separation Module (Waters, Milford, MA) equipped with an autoinjector, a 2996 photodiode array detector (PAD) and Empower software and Atlantis C_{18} (5 μm, 20 × 4.6 mm) column (Waters) and a 4.6 × 2.0 mm guard column were used for separation at 28 °C with a flow rate of 0.5 ml/min. Elution of desulfoglucosinolates from the HPLC column was performed by a gradient system of water (**A**) and acetonitrile/water (20:80, v/v, **B**). The total running time was 35 min with a gradient as follows: 98% **A** for 1 min, then in 35 min to 75% **A**, and in 1 min back to 98% **A** and re-equilibrated for 10 min. Eluent was monitored by diode array detection between 200 and 400 nm (2 nm interval). Desulfoglucosinolates were identified by comparison of retention time and UV spectral characteristics to desulfoglucosinolates of pure standards of sinigrin, glucotropaeolin, the typical glucosinolate pattern of BCR-367 rapeseed (Linsinger, Kristiansen, Beloufa, Schimmel, & Pauwels, 2001) and purified extracts from white cabbage, red cabbage and mashua (*Tropaeolum tuberosum* Ruiz & Pavon). Detection was carried out at 229 nm. Molar concentrations of individual glucosinolates and relative response factors (Brown, Tokuhisa, Reichelt, & Gershenzon, 2003; Linsinger et al., 2001) were used to correct for absorbance differences between the internal standard (sinigrin) and the other components of the extract. Total GLs were expressed as μmol g^{-1} DW and determined by adding up all individual GLs (Linsinger et al., 2001).

2.6. Myrosinase activity (thioglucoside glucohydrolase, EC 3.2.3.1)

The methodology described by Verkerk and Dekker (2004) with some modifications was used. Maca hypocotyls were homogenised with 100 mM (pH 6.0) phosphate buffer in a 1:10 ratio (w/v) in a commercial blender. This solution was filtered to remove the solid

particles and incubated in a water bath at 40 °C for 2 h to hydrolyse the endogenous glucosinolates. Five millilitre of hydrolysed extract were treated with 1 ml of 6 mM sinigrin at 40 °C for 20 min. The reaction was stopped with 12 ml of hot methanol (~75 °C) for 10 min at 75 °C. The extract was centrifuged at 6000 *g* for 15 min. The extract was purified, desulfated and analysed by HPLC as detailed in Sections 2.3 and 2.4. Myrosinase activity was expressed as amount of transformed sinigrin in mmol min^{-1} g^{-1} DW.

2.7. Statistical analysis

Three replicates were used in all analysis. Results were processed by one-way analysis of variance. Duncan's test was performed to account for mean differences amongst different treatments. Differences at $p < 0.05$ were considered as significant. SPSS software for Windows 14.0 (SPSS, Chicago, IL) was used to perform all statistical analyses.

3. Results and discussion

3.1. Identification and quantification of GLs at harvest in three maca ecotypes

Six different glucosinolates were identified in the three coloured maca ecotypes (Fig. 1). These glucosinolates corresponded to three aromatic: 4-hydroxybenzyl (glucosinalbin), benzyl (glucotropaeolin) and 3-methoxybenzyl (glucolimnanthin); one aliphatic: 5-methylsulfinylpentyl (glucoalyssin) and two indolic: 4-hydroxy-3-indolylmethyl (4-hydroxyglucobrassicin) and 4-methoxy-3-indolylmethyl (4-methoxyglucobrassicin) (Table 1). The presence of glucoalyssin was confirmed with the white cauliflower glucosinolate profile (Cieślik, Leszczyńska, Filipiak-Florkiewicz, Sikora, & Pisulewski, 2007). This glucosinolate is important in *Lepidium* (Fahey et al., 2001). Glucotropaeolin was confirmed with an external standard. Glucolimnanthin (3-methoxybenzyl) is the second most important glucosinolate in maca (peak 5, Fig. 1) as previously reported by Piacente, Carbone, Plaza, Zampelli, and Pizza (2002) and Clément et al. (2010). The presence of glucoaubrietin was discarded through assays carried out with *Tropaelum tuberosum* Ruiz & Pavon as reported by Ramallo et al. (2004) who highlighted that this is the most important glucosinolate in mashua. 4-Hydroxyglucobrassicin was confirmed with the retention times and absorption spectra of the glucosinolates found in Rapeseed BCR-367 and extracts of red cabbage and white cauliflower(Oerlemans, Barrett, Suades, Verkerk, & Dekker, 2006). 4-Methoxyglucobrassicin was confirmed with the red cabbage and white cauliflower (Volden, Borge, Hansen, Wicklund, & Bengtsson, 2009) profiles. Li et al. (2001) identified and quantified GLs in different parts of maca plants and processed products. Thus, in the hypocotyl of fresh yellow maca eight glucosinolates were reported: 5-methylsulfinylpentyl glucosinolate (glucoalyssin), 4-hydroxybenzyl glucosinolate (glucosinalbin), 3-hydroxybenzyl glucosinolate (tentative identification), 4-pentenyl glucosinolate (gluco- brassicanapin), benzyl glucosinolate (glucotropaeolin), indol-3-ylmethyl glucosinolate (glucobrassicin), 4-methoxybenzyl glucosinolate (glucoaubrietin) and 4-methoxyindol-3-ylmethyl glucosinolate (4-methoxyglucobrassicin). Recently, Clément et al. (2009) identified and quantified different GLs in yellow, pink, violet and lead maca ecotypes. These corresponded to: glucotropaeolin, glucolimnathin (alternatively glucoaubrietin), glucosinalbin, 4-hydroxyglucobrassicin, glucoalyssin and glucoraphanin. Dini et al. (2002) identified glucotropaeolin and glucolimnanthin in maca flour and suggested the use of these two GLs as chemotaxonomic markers for this specie since this combination of GLs does not happen in other plants of the *Brassicaceae* family. However, it is clear that the most important GL corresponds to glucotropaeolin. Clément et al. (2009) reported that 80–90% of the total GLs in maca corresponded to glucotropaeolin.

The quantification of total GLs in the yellow, red and black ecotypes corresponded to 36.2, 34.9 and 31.4 mmol kg^{-1} DW (corresponding to 30.4, 28.2 and 25.1 mmol kg^{-1} FW), respectively. The sum of the aromatic GLs represented on average 99% of the total GL content (35.9, 34.5 and 31.1 mmol kg^{-1} DW) from which glucotropaeolin was the most abundant in the three ecotypes (29.1, 28.4, 25.2 mmol kg^{-1} DW) representing around 80% of the total GLs. No significant differences ($p > 0.05$) in total GLs, aromatic GLs and glucotropaeolin were found for the three maca ecotypes. The work of Clément et al. (2010) reported differences in GLs related to the colour of the maca ecotype. However, for the yellow maca ecotype, GLs were higher than the ones reported by Li et al. (2001) (25.7 and 16.9 mmol kg^{-1} DW for total GLs and glucotropaeolin, respectively). Clément et al. (2010) reported values of 37.2, 36.4 and 31.4 mmol kg^{-1} DW for total GLs, aromatic GLs and glucotropaeolin, respectively. These values were similar to the ones reported in this work. Our results demonstrate that maca is an important source of aromatic glucosinolates. Currently, research on benzyl isothiocyanate and its precursor benzyl glucosinolate as cancer chemopreventives is under way (Higdon, Delage, Williams, & Dashwood, 2007). Contents of the alkylthioalkyl GL (glucoalyssin) of 0.25, 0.23 and 0.28 mmol kg^{-1} DW were found for the yellow, red and black ecotypes, respectively. These values represented from 0.6% to 0.9% of the total GLs, and the indole GLs represented from 0.2% to 0.3% of the total GLs. Clément et al. (2010) reported contents of alkylthioalkyl GL (glucoalyssin and glucoraphanin) of 0.42, 0.71, 2.0 and 0.49 mmol kg^{-1} DW (1–6.5% of the total GLs) for yellow, pink, violet and lead maca ecotypes. Indolic GLs were found in minor quantities (0.06–0.09 mmol kg^{-1} DW), compared to the values of 0.15–0.26 mmol kg^{-1} DW found by Clément et al. (2010). The differences found in the profile and content of GLs between this work and the one of Clément et al. (2010) might be related to the fact that they worked with lab accessions and experimental cultivation conditions. The variation in the quantity and profile of GLs is attributed to genetic and environmental factors, age and parts of the plant, predator attacks, temperature, water stress, soil type and agronomic factors (Velasco, Cartea, Gonzáles, Vilar, & Ordás, 2007). The cultivation practices in this work corresponded to the cultural practices of the people from the local communities.

3.2. GL profiles and contents for the three maca ecotypes during preharvest

The pre-harvest period is considered as the glucosinolate and other bioactive compound biosynthetic stage in the hypocotyls. The biosynthesis of glucosinolates can be divided in three phases: (i) formation of the 2-oxo acid with an additional methylene group (ii) formation of the core structure of the glucosinolate and (iii) transformation of the R group. The involved genes have been identified in the three biosynthetic phases (Halkier & Gershenzon, 2006). Only a few studies exist in sprouts but rarely during preharvest.

The total content of glucosinolates increased during the growth of the hypocotyls (Table 2). Significant differences ($p < 0.05$) were observed for the different pre-harvest time points. Ninety days before harvest and at harvest, values of 11.3 and 36.2, 10.5 and 34.9, 11.2 and 31.4 mmol kg^{-1} DW were obtained for the yellow, red and black hypocotyls, respectively. During the pre-harvest stage, only a small quantity of glucoalyssin was found. Most of the total GLs (99%) corresponded to the aromatic type (glucosinalbin + glucotropaeolin + glucolimnantin) of which 75.6–87.4% corresponded to glucotropaeolin. No indolic GLs were detected during

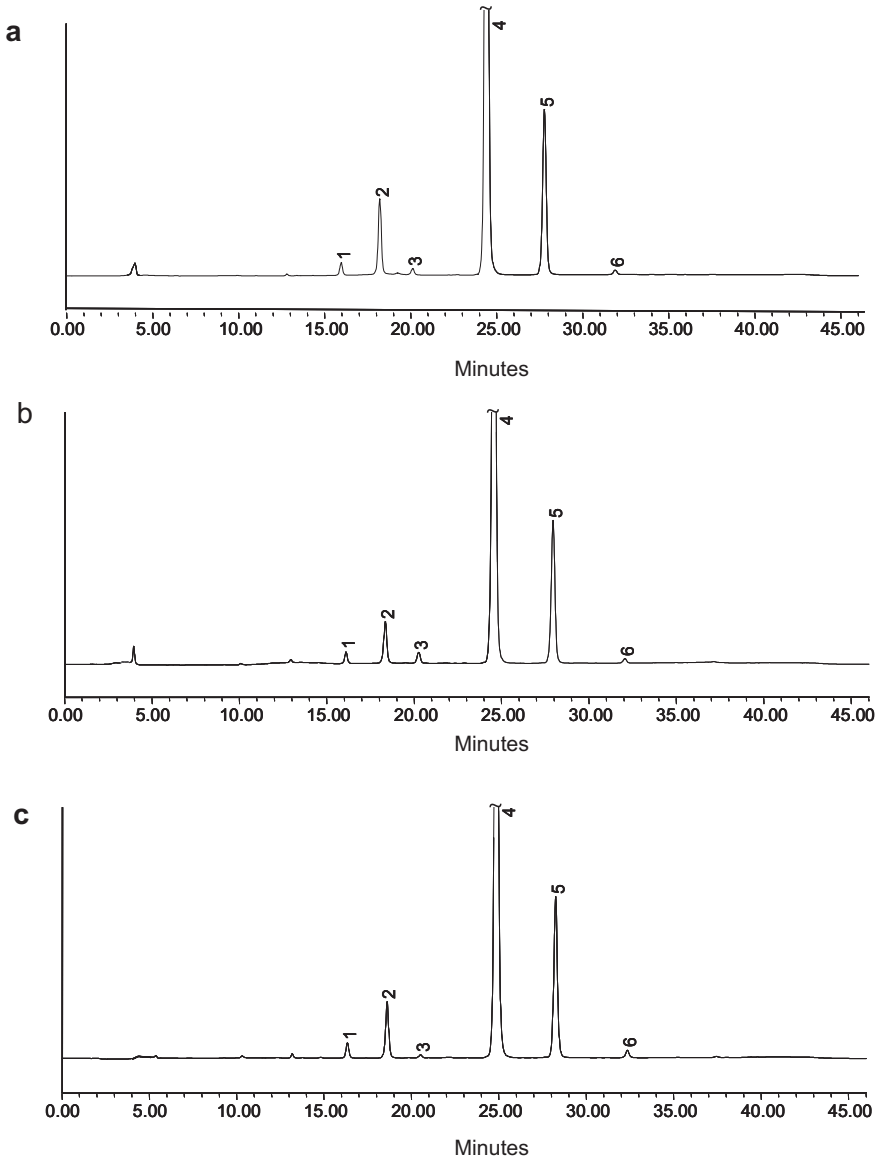

Fig. 1. HPLC glucosinolate profiles for the yellow, red and black maca ecotypes at harvest (fresh maca): (a) yellow (b) red and (c) black. (1) Glucoalyssin, (2) glucosinalbin, (3) 4-hydroxyglucobrassicin, (4) glucotropaeolin, (5) glucolimnanthin and (6) 4-methoxyglucobrassicin.

Table 1
Glucosinolate content at harvest time for three maca ecotypes.

Systematic name	Trivial name	Class	Yellow (mmol kg^{-1} DW)[A]	Red (mmol kg^{-1} DW)[A]	Black (mmol kg^{-1} DW)[A]
5-Methylsulfinylpentyl glucosinolate	Glucoalyssin	Aliphatic	0.25 ± 0.01[a]	0.23 ± 0.01[a]	0.28 ± 0.01[b]
4-Hydroxybenzyl glucosinolate	Glucosinalbin	Aromatic	1.03 ± 0.08[b]	0.58 ± 0.01[a]	0.69 ± 0.02[a]
Benzyl glucosinolate	Glucotropaeolin	Aromatic	29.1 ± 1.67[a]	28.4 ± 0.42[a]	25.2 ± 0.75[a]
3-Methoxybenzyl glucosinolate	Glucolimnanthin	Aromatic	5.77 ± 0.73[a]	5.51 ± 0.02[a]	5.18 ± 0.16[a]
4-Hydroxy-3-indolylmethyl glucosinolate	4-Hydroxyglucobrassicin	Indolyl	0.04 ± 0.01[a]	0.07 ± 0.07[a]	0.01 ± 0.00[a]
4-Methoxy-3-indolylmethyl glucosinolate	4-Methoxyglucobrassicin	Indolyl	0.02 ± 0.01[a]	0.02 ± 0.01[a]	0.06 ± 0.00[a]
		Total	36.2 ± 2.12[a]	34.9 ± 0.56[a]	31.4 ± 0.94[a]

Means within a row with the same superscript letter are not significantly different ($p > 0.05$).
[A] Results are means ± SD ($n = 3$).

growth. No significant differences ($p > 0.05$) in total GLs were observed in the three maca ecotypes for the different evaluated pre-harvest time points. Velasco et al. (2007) reported for kale leaves (*Brassica oleracea* Acephala group) taken 30, 90, 180 and 300 after cultivation that GLs increased (8.45, 9.31, 41.03 and 44.63 μmol g^{-1} DW respectively). The most important GL, sinigrin, showed a similar trend to total GLs. The content of glucobrassicin increased during the first three stages of plant development (3.45, 4.20 and 12.6 mmol kg^{-1} DW, respectively), but then decreased to 7.48 mmol kg^{-1} DW at 300 days. The increase in the total GL content is explained with *de novo* synthesis theory. It is also confirmed that indole glucosinolates are more sensitive to environmental factors than aliphatic glucosinolates.

Table 2
Glucosinolate content in maca hypocotyls during pre-harvest (mmol kg^{-1} DW).

Glucosinolates	Pre-harvest (days)				Harvest
	90	45	30	15	
Yellow					
Glucoalyssin	0.11 ± 0.00^{A}	0.10 ± 0.01^{A}	0.14 ± 0.01^{AB}	0.18 ± 0.03^{B}	0.25 ± 0.01^{B}
Glucosinalbin	0.37 ± 0.01^{AB}	0.31 ± 0.02^{A}	0.45 ± 0.06^{AB}	0.60 ± 0.09^{B}	1.03 ± 0.08^{C}
Glucotropaeolin	8.53 ± 0.25^{A}	17.5 ± 1.72^{B}	17.8 ± 0.82^{B}	25.1 ± 0.57^{C}	29.1 ± 1.67^{C}
Glucolimnanthin	2.26 ± 0.07^{A}	2.76 ± 0.27^{A}	3.74 ± 0.18^{B}	4.91 ± 0.09^{C}	5.77 ± 0.73^{D}
4-Hydroxyglucobrassicin	ND	ND	ND	ND	0.04 ± 0.01^{A}
4-Methoxyglucobrassicin	ND	ND	ND	ND	0.02 ± 0.01^{A}
Total	11.3 ± 0.33^{Aa}	20.7 ± 2.02^{Ba}	22.1 ± 1.06^{Bab}	30.7 ± 0.78^{Cb}	36.2 ± 2.12^{Da}
Red					
Glucoalyssin	0.12 ± 0.03^{AB}	0.03 ± 0.01^{A}	0.17 ± 0.02^{BC}	0.11 ± 0.03^{AB}	0.23 ± 0.01^{C}
Glucosinalbin	0.38 ± 0.02^{AB}	0.23 ± 0.01^{A}	0.44 ± 0.06^{BC}	0.60 ± 0.06^{D}	0.58 ± 0.01^{C}
Glucotropaeolin	7.97 ± 0.33^{A}	16.0 ± 0.72^{B}	19.9 ± 0.49^{C}	26.1 ± 0.39^{D}	28.4 ± 0.42^{E}
Glucolimnanthin	2.07 ± 0.07^{A}	2.29 ± 0.09^{A}	4.70 ± 0.14^{C}	3.04 ± 0.06^{B}	5.51 ± 0.02^{D}
4-Hydroxyglucobrassicin	ND	ND	ND	ND	0.07 ± 0.07^{A}
4-Methoxyglucobrassicin	ND	ND	ND	ND	0.02 ± 0.01^{A}
Total	10.5 ± 0.45^{Aa}	18.6 ± 0.84^{Ba}	25.2 ± 0.71^{Cb}	29.9 ± 0.54^{Db}	34.9 ± 0.56^{Ea}
Black					
Glucoalyssin	0.13 ± 0.03^{A}	0.09 ± 0.03^{A}	0.11 ± 0.01A	0.15 ± 0.00^{A}	0.28 ± 0.01^{B}
Glucosinalbin	0.29 ± 0.03^{A}	0.35 ± 0.03^{AB}	0.45 ± 0.03^{B}	0.39 ± 0.00^{AB}	0.69 ± 0.02^{C}
Glucotropaeolin	8.88 ± 0.63^{A}	14.6 ± 0.39^{B}	17.3 ± 0.13^{C}	21.9 ± 0.25^{D}	25.2 ± 0.75^{E}
Glucolimnanthin	1.93 ± 0.14^{A}	2.26 ± 0.11^{A}	2.86 ± 0.03^{B}	3.76 ± 0.05^{C}	5.18 ± 0.16^{D}
4-Hydroxyglucobrassicin	ND	ND	ND	ND	0.01 ± 0.00^{A}
4-Methoxyglucobrassicin	ND	ND	ND	ND	0.06 ± 0.00^{A}
Total	11.2 ± 0.84^{Aa}	17.3 ± 0.55^{Ba}	20.8 ± 0.21^{Ca}	26.2 ± 0.30^{Da}	31.43 ± 0.94^{Ea}

ND: not detected. *Results are means ± SD ($n = 3$).
Means within a row with the same superscript capital letter are not significantly different ($p > 0.05$), columns with the same superscript small letter are not significantly different ($p > 0.05$).

3.3. GL profiles and contents for the three maca ecotypes during post-harvest drying

A significant increase in GL content ($p < 0.05$) was observed during the first 15 days of post-harvest drying for the yellow, red and black hypocotyls (26.3%, 36.7% and 38.5%), respectively. No significant differences ($p > 0.05$) were observed between 15 and 30 days of post-harvest drying. Increases were similar for the sum of aromatic GLs and glucotropaeolin (Table 3). This increase might be due to the stress provoked by the conditioning of the hypocotyls before drying. Thus, the heat generated by respiration of the whole plant (hypocotyls plus leaves), the piling up in the field and the packaging in 50-kg net sacks generated a modified atmosphere and thus might have accelerated the metabolism during the first four days. Another event is the translocation of the GLs from the leaves to the hypocotyls. However, during this period a small loss of GLs might be expected due to myrosinase activity, since the hypocotyls are exposed to extreme temperatures of −5 °C to −10 °C during the night and 15 °C during the day. During freezing and thawing, cells are broken, and the enzyme and the substrate (GLs), which were previously in different compartments, come into contact. These losses attributed to myrosinase activity during this period are insignificant compared to the increment caused by the stress induced during the post-harvest handling activities and translocation of GLs from the leaves to the hypocotyls. In broccoli stored 7 days at 10 °C in different atmospheres, different behaviours were observed. When stored in air and 0.5% O_2 + 20% CO_2, the total GLs increased by 42% and 21%, respectively, whilst at 20% CO_2, the total content of GLs decreased by 15% (Hansen, Moller, & Cantwell de Trejo, 1995). An expected increase in the level of indole glucosinolates was observed in cabbage and broccoli after wounding and 48-h storage at ambient conditions. White cabbage increased 15 times the content of 4-methoxy- and 1-methoxy-3-indolylmethyl glucosinolates, whilst in broccoli increments of 3.5 and 2-folds for 4-hydroxy- and 4-methoxy-3-indolylmethyl glucosinolates were observed, respectively. In these cases, the GL hydrolysis induced by myrosinase activity seems to be counterbalanced by the post-harvest increase of the indolyl glucosinolates due to an induced stress (Verkerk et al., 2001). According to Hodges, Munro, Forney, and McRae (2006), the increases in the content of gluconapin and glucobrassicin in cauliflower at 0 °C in modified atmosphere conditions might be related to metabolic changes associated with natural senescence and/or induced stress. Kale leaves (*B. oleracea* Acephala group) should be preferentially consumed between 180 and 300 days after cultivation since at 390 days, translocation of GLs from the leaves to the flower buds occurs (Velasco et al. 2007).

Between 30 and 45 days of post-harvest drying, a significant decrease ($p < 0.05$) of GLs was observed. After 45 days of post-harvest drying, gradual losses were observed. Between 60 and 90 days, the decrease of total GLs was significantly lower for the three ecotypes ($p < 0.05$). This trend for total GLs, aromatic GLs and glucotropaeolin is observed for the three ecotypes. Losses observed between 30 and 60 days of post-harvest drying might be related to the action of myrosinase, given the disruption of cells due to the freezing–thawing cycles. Oscillating temperatures between −10 to 15 °C and the humidity of the hypocotyls (40–55%) is sufficient for the enzyme to be active. On the other hand, between 75 and 90 days of post-harvest drying, the humidity of the hypocotyls (12–30%) restricted the action of myrosinase. At the end of the post-harvest drying (90 days), the total GLs content for the yellow, red and black maca ecotypes were 22.6, 27.7 and 15.0 mmol kg^{-1} DW, respectively. Thus, losses of total GLs of 37.6, 20.4 and 52.4% were observed for the yellow, red and black maca ecotypes, respectively.

3.4. Myrosinase activity during pre-harvest, at harvest and post-harvest drying

Myrosinase (thioglucoside glucohydrolase, EC 3.2.1.147, formerly EC 3.2.3.1) catalyses the hydrolysis of glucosinolates in plants of the order Brassicales after tissue damage (Travers-Martin, Kuhlmann, & Müller, 2008).

Table 3
Glucosinolate content in maca hypocotyls during post-harvest drying (mmol kg^{-1} DW).

Glucosinolates	Harvest	Post-harvest (days)					
		15	30	45	60	75	90
Yellow							
Glucoalyssin	0.25 ± 0.01^{AB}	0.40 ± 0.03^{B}	0.21 ± 0.02^{A}	0.19 ± 0.02^{A}	0.21 ± 0.10^{A}	0.23 ± 0.04^{A}	0.15 ± 0.02^{A}
Glucosinalbin	1.03 ± 0.08^{CD}	1.26 ± 0.08^{D}	$0.86 \pm 0.05B^{C}$	0.55 ± 0.02^{A}	0.70 ± 0.16^{AB}	0.84 ± 0.03^{ABC}	0.75 ± 0.00^{ABC}
Glucotropaeolin	29.1 ± 1.67^{B}	35.9 ± 1.53^{C}	37.9 ± 1.53^{C}	24.7 ± 1.43^{AB}	22.2 ± 2.93^{A}	18.1 ± 1.16^{A}	18.4 ± 0.37^{A}
Glucolimnanthin	5.77 ± 0.73^{B}	8.00 ± 0.35^{C}	5.62 ± 0.24^{B}	4.50 ± 0.29^{AB}	4.54 ± 0.65^{AB}	4.53 ± 0.32^{AB}	3.28 ± 0.05^{A}
4-Hydroxyglucobrassicin	0.04 ± 0.01^{A}	0.06 ± 0.03^{A}	ND	ND	ND	ND	ND
4-Methoxyglucobrassicin	0.02 ± 0.01^{A}	0.03 ± 0.01^{A}	ND	ND	ND	ND	ND
Total	36.2 ± 2.12^{BCa}	45.7 ± 2.03^{Da}	44.6 ± 1.85^{CDa}	29.9 ± 1.76^{ABa}	27.6 ± 3.84^{Aa}	23.7 ± 1.55^{Aab}	22.6 ± 0.44^{Ab}
Red							
Glucoalyssin	0.23 ± 0.01^{B}	0.35 ± 0.02^{C}	0.27 ± 0.03^{BC}	0.12 ± 0.03^{A}	0.21 ± 0.02^{B}	0.30 ± 0.02^{BC}	0.24 ± 0.02^{B}
Glucosinalbin	0.58 ± 0.01^{A}	1.21 ± 0.02^{C}	1.00 ± 0.07^{B}	0.62 ± 0.06^{A}	0.91 ± 0.06^{B}	0.88 ± 0.02^{B}	0.66 ± 0.02^{A}
Glucotropaeolin	28.4 ± 0.42^{B}	39.6 ± 0.15^{C}	40.9 ± 2.47^{C}	22.91 ± 2.00^{A}	20.00 ± 0.42^{A}	21.1 ± 0.08^{A}	22.1 ± 0.29^{A}
Glucolimnanthin	5.51 ± 0.02^{C}	6.38 ± 0.03^{D}	6.63 ± 0.40^{D}	3.51 ± 0.32^{A}	3.67 ± 0.10^{A}	5.54 ± 0.01^{C}	4.65 ± 0.09^{B}
4-Hydroxyglucobrassicin	0.07 ± 0.07^{A}	0.04 ± 0.01^{A}	ND	ND	ND	ND	ND
4-Methoxyglucobrassicin	0.02 ± 0.01^{A}	0.05 ± 0.02^{A}	ND	ND	ND	ND	ND
Total	34.9 ± 0.56^{Ba}	47.6 ± 0.25^{Ca}	48.8 ± 2.98^{Ca}	27.2 ± 2.42^{Aa}	24.8 ± 0.60^{Aa}	27.8 ± 0.13^{Ab}	27.7 ± 0.41^{Ac}
Black							
Glucoalyssin	0.28 ± 0.01^{C}	0.26 ± 0.02^{C}	0.22 ± 0.00^{BC}	0.23 ± 0.01^{BC}	0.13 ± 0.06^{AB}	0.18 ± 0.01^{ABC}	0.08 ± 0.01^{A}
Glucosinalbin	$0.69 \pm 0.02B^{CD}$	0.86 ± 0.06^{D}	0.81 ± 0.00^{CD}	0.89 ± 0.03^{D}	0.52 ± 0.10^{AB}	0.63 ± 0.06^{BC}	0.32 ± 0.02^{A}
Glucotropaeolin	25.2 ± 0.75^{C}	36.7 ± 2.51^{D}	34.9 ± 0.18^{D}	26.5 ± 0.75^{C}	18.4 ± 2.94^{B}	13.9 ± 1.57^{AB}	11.8 ± 0.85^{A}
Glucolimnanthin	5.18 ± 0.16^{B}	5.65 ± 0.38^{B}	5.48 ± 0.02^{B}	5.37 ± 0.17^{B}	3.12 ± 0.55^{A}	3.24 ± 0.36^{A}	2.77 ± 0.23^{A}
4-Hydroxyglucobrassicin	0.01 ± 0.00^{A}	0.01 ± 0.00^{A}	ND	ND	ND	ND	ND
4-Methoxyglucobrassicin	0.06 ± 0.00^{C}	0.03 ± 0.00^{B}	0.02 ± 0.00^{A}	ND	ND	ND	ND
Total	31.4 ± 0.94^{Ba}	43.53 ± 2.93^{Ca}	41.4 ± 0.21^{Ca}	33.0 ± 0.97^{Ba}	22.2 ± 3.66^{Aa}	18.0 ± 2.00^{Aa}	15.0 ± 1.12^{Aa}

* Results are means ± SD ($n = 3$).
ND: not detected.
Means within a row with the same superscript capital letter are not significantly different ($p > 0.05$), columns with the same superscript small letter are not significantly different ($p > 0.05$).

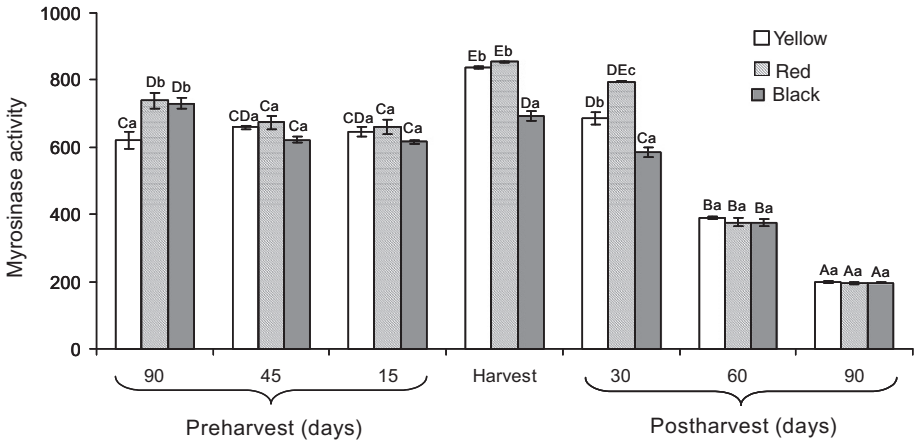

Fig. 2. Myrosinase activity (nmol min^{-1} g^{-1} DW) for three maca ecotypes during pre-harvest, at harvest and during post-harvest drying of maca hypocotyls. * Results are means ± SD ($n = 3$). Different capital letters stand for significant differences ($p < 0.05$) for a maca ecotype during the whole evaluation (pre-harvest, harvest and post-harvest drying). Different lower case letters stand for significant differences ($p < 0.05$) amongst the three ecotypes at a given time point.

Before harvesting, myrosinase activity ranged from 622 to 739 nm min^{-1} g^{-1} DW (Fig. 2). Higher myrosinase activity was found 90 days before harvest for the red and black ecotypes. Similar myrosinase activity was found 45 and 15 days prior to harvest for the three ecotypes ($p > 0.05$). At harvest, myrosinase activity considerably increased in the yellow and red hypocotyls (838 and 854 nm min^{-1} g^{-1} DW) compared to the black (693 nm min^{-1} g^{-1} DW) ecotype. Myrosinase activity (nm min^{-1} g^{-1} FW) in the broccoli seed of the calabrese cultivar was ~7, increased to 8.5 after 2 days of germination and decreased to 4.5 on day 3 of germination. It stayed in the 4–6 range up to 14 days of germination (Williams, Critchley, Pun, Nottingham, & O'Hare, 2008). According to Travers-Martin et al. (2008) myrosinase is present in different forms (soluble and insoluble) and its activity is dependent on the substrate and plant part.

Significant losses of myrosinase activity ($p < 0.05$) were observed during the post-harvest drying stage. After 30 days, the observed values (% decrease) corresponded to: 686 (18.1%), 791 (6.8%) and 585 (15.5%) nm min^{-1} g^{-1} DW for the yellow, red and black ecotypes, respectively. After 60 days of post-harvest drying, these values were of: 390 (53.4%), 376 (56%) and 376 (45.8%) nm min^{-1} g^{-1} DW, for the yellow, red and black ecotypes, respectively. The final values obtained at the end of the post-harvest drying process corresponded to: 199 (76.1%), 194 (77.3%) and 196 (71.8%) nm min^{-1} g^{-1} DW, for the yellow, red and black ecotypes, respectively. After 60 days of post-harvest drying, a

lower myrosinase activity was observed and it was correlated with a smaller loss of total GLs between 60 and 90 days of post-harvest drying (Table 3).

Myrosinase activity during maca drying seemed to be related to the water loss, water availability, structure of the enzyme and factors that conditioned its activity. The presence of several ionic bonds, disulphide bridges and H-bonds promotes stability of myrosinase in the extracellular environment where the enzyme works after tissue damage. Myrosinases are highly glycosylated with carbohydrates representing up to 20% of their molecular mass (Halkier & Gershenzon, 2006). Several works indicate that the glucosinolate/myrosinase system is modified during processing of *Brassica* vegetables, due to the partial or total inactivation of the enzyme. In addition, the glucosinolate concentrations and residual myrosinase activity in cabbage are dependent on the method and duration of processing (Rungapamestry, Duncan, Fuller, & Ratcliffe, 2006). Jones, Faragher, and Winkler (2006) reported that drying of intact broccoli at 50–65 °C maintained glucosinolates and myrosinase activity. It was only when the product was rehydrated that glucosinolates were hydrolysed (Rosa, Heaney, Fenwick, & Portas, 1997).

4. Conclusions

Maca is an important source of glucosinolates mainly of the aromatic type (glucotropaeolin). In total, six glucosinolates were identified in the yellow, red and black ecotypes studied. These glucosinolates corresponded to 5-methylsulfinylpentyl, 4-hydroxybenzyl, benzyl, 3-methoxybenzyl, 4-hydroxy-3-indolylmethyl and 4-methoxy-3-indolylmethyl.

The glucosinolate profile and content for the three analysed ecotypes were similar. They gradually and significantly increased 90 days before harvest. During post-harvest drying (15–30 days) at ambient conditions (−10 to 15 °C, 70–90% relative humidity), the three analysed maca ecotypes displayed an important increase of total and aromatic GLs. After 45 days and at the end of this process, losses from 20% to 50% of total GLs were correlated to the decrease in myrosinase activity.

This artisanal and traditional drying technique in the Peruvian Andes must be revised due to the high losses of GLs. An alternative approach that includes drying in ambient conditions for 15 days followed by blanching to inactivate myrosinase and concluded with hot air drying might be evaluated. Products with a significant higher added value (much higher GL content) can be obtained.

Acknowledgements

The authors are thankful to Ecoandino s.a.c for the maca material. This research was supported by the Consejo Nacional de Ciencia y Tecnología (CONCYTEC), Peru.

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