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Bioactive maca (*Lepidium meyenii*) alkamides are a result of traditional Andean postharvest drying practices

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ABSTRACT

Maca, *Lepidium meyenii* Walpers (Brassicaceae), is an annual herbaceous plant native to the high plateaus of the Peruvian central Andes. Its underground storage hypocotyls have been a traditional medicinal agent and dietary staple since pre-Columbian times. Reported properties include energizing and fertility-enhancing effects. Published reports have focused on the benzylalkamides (macamides) present in dry hypocotyls as one of the main bioactive components. Macamides are secondary amides formed by benzylamine and a fatty acid moiety, with varying hydrocarbon chain lengths and degree of unsaturation. Although it has been assumed that they are usually present in fresh undamaged tissues, analyses show them to be essentially absent from them. However, hypocotyls dried by traditional Andean postharvest practices or industrial oven drying contain up to 800 μg g^{-1} dry wt (2.3 μmol g^{-1} dry wt) of macamides. In this study, the generation of macamides and their putative precursors were studied during nine-week traditional drying trials at 4200 m altitude and in ovens under laboratory conditions. Freeze–thaw cycles in the open field during drying result in tissue maceration and release of free fatty acids from storage and membrane lipids up to levels of 1200 μg g^{-1} dry wt (4.3 μmol g^{-1} dry wt). Endogenous metabolism of the isothiocyanates generated from glucosinolate hydrolysis during drying results in maximal benzylamine values of 4300 μg g^{-1} dry wt (40.2 μmol g^{-1} dry wt). Pearson correlation coefficients of the accumulation profiles of benzylamine and free fatty acid to that of macamides showed good values of 0.898 and 0.934, respectively, suggesting that both provide sufficient substrate for amide synthesis during the drying process.

1. Introduction

Maca (*Lepidium meyenii* Walpers or *Lepidium peruvianum* Chacón), an annual herbaceous plant of the Brassicaceae family, is native to the central Andes. It is the only reported species of the genus *Lepidium* displaying a fused hypocotyl and taproot forming an underground storage organ which is well adapted to the harsh climate of the high-altitude central Andean plateau. The plant, also mentioned as a "lost crop of the Incas" (NRC, 1989), has been cultivated and used for food and medicinal purposes since pre-Columbian times. It has gained attention in the past two decades due to reports on medicinal properties which make it a good candidate for the nutraceutical market (Canales et al., 2000; Dini et al., 1994). As a consequence, reported Peruvian exports of dried and processed maca rose almost fourfold in the past decade, to over USD 10 million in 2013 (SCIICEX, 2013).

Maca presents three major phenotypes, red, yellow and black (Fig. 1), based on their hypocotyl and stem coloration. As in most Brassicaceae, glucosinolates accumulate in its tissues, of which benzylglucosinolate (**1**) (Fig. 2) is the main product. Depending on the chemotype, lesser amounts of the 3- or 4-, hydroxy or methoxylated benzyl derivatives (**14**–**17**) (Fig. S1) and of tryptophan-derived compounds (**18**–**20**) can be present (Fig. S1) (Li et al., 2001; Piacente et al., 2002; Clément et al., 2010; Yábar et al., 2011). Published reports suggest that differences in the chemical composition of the phenotypes are associated with the reported biological effects or medical target for which these different types can be used. For example, black maca is useful in stimulating sperm count (Gonzales et al., 2006) while red maca is most useful for the treatment of benign prostate hyperplasia (Gonzales et al., 2005). Black maca has been also reported to increase memory and learning in mice (Rubio et al., 2006, 2007). Other reported properties which may not be related to phenotype include increases in female fertility and libido (Ruiz-Luna et al., 2005) and a stimulatory effect on the central nervous system through

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Fig. 1. Maca hypocotyls of three characteristic phenotypes, yellow, red and black. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

inhibition of mammalian fatty acid amide hydrolases (FAAH) and potentiation of the endocannabinoid system (Pino-Figueroa et al., 2011). Wang and coworkers (2007) have published a comprehensive summary on the reported biological and pharmacological properties of maca.

The drying of plant tissues, be it during seed production or by human action as in postharvest processing, is a complex biochemical process in which the degree of tissue damage and ensuing reactions during desiccation determine the composition and viability of the product. Maca is routinely exported in the form of flour obtained from field or oven-dried hypocotyls or from lyophilized fresh material. The difference in composition between these products is, as expected, significant and depends on the degree of activity that various hydrolytic enzymes have had, especially that of the endogenous thioglucosidases (myrosinases). Therefore, one criterion regarded as important for the quality of the dried product is the levels of glucosinolates, which are expected to resemble as much

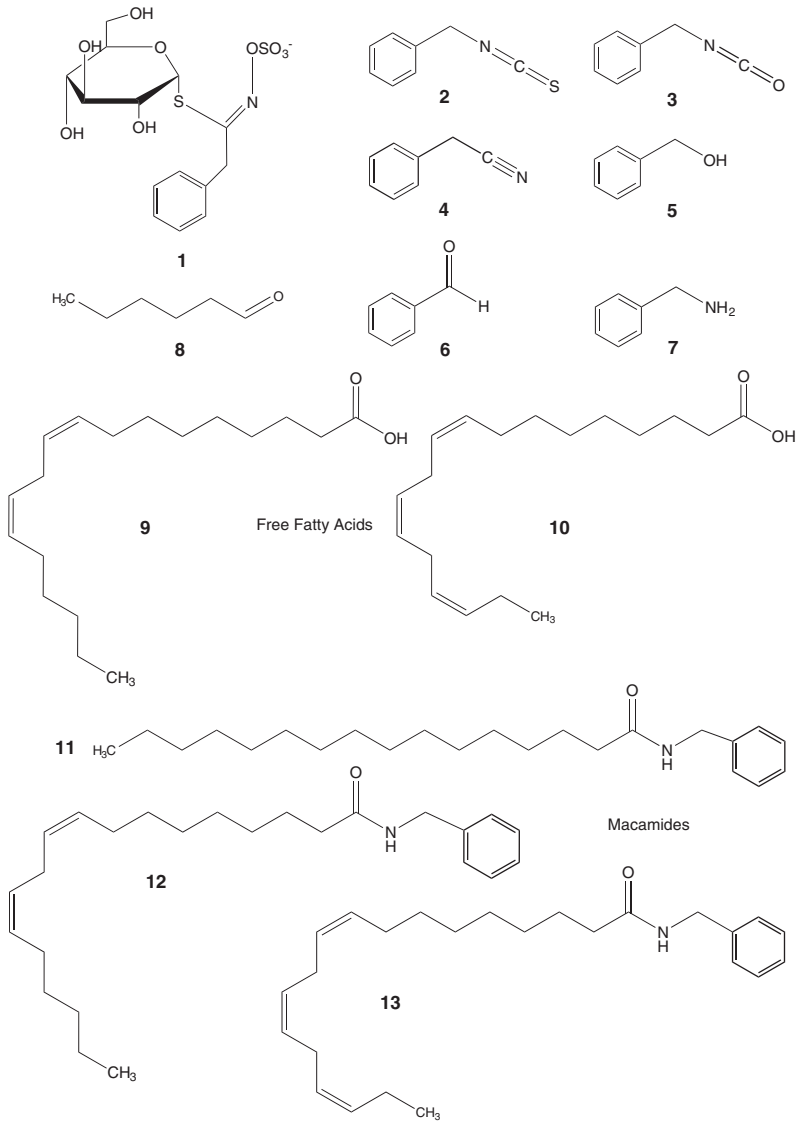

Fig. 2. The main maca glucosinolate and metabolites analysed in this study. BGl (**1**): benzylglucosinolate, BITC (**2**): benzyl isothiocyanate, BIOC (**3**): benzyl isocyanate, BCN (**4**): benzyl nitrile, BOH (**5**): benzyl alcohol, BCHO (**6**): benzaldehyde, BNH_2 (**7**): benzylamine, hexanal (**8**), FFA 18:2 (**9**): linoleic acid (**9**), FFA 18:3 (**10**): linolenic acid, MAC 16 (**11**): N-benzyl hexadecanamide, MAC 18:2 (**12**): N-benzyl-(9Z,12Z)-octadecadienamide, MAC 18:3 (**13**): N-benzyl-(9Z,12Z,15Z) octadecatrienamide.

as possible the original values (Xing-Hao et al., 2014) and of their hydrolytic metabolites, such as aromatic isothiocyanates and their more stable aldehydes, nitriles and alcohols. Most maca flour is usually obtained from hypocotyls that have been dried in the open air on site at least for a few weeks prior to oven drying at 60 °C. The traditional open-field drying takes usually over two months and involves exposure to the extreme temperature cycles and strong light conditions and low atmospheric pressure typical of the high altitude environment (>3500 m) where maca grows. The variability in environmental conditions during drying, and in the handling of the product, which involves considerable bruising of the tissues, means that one can expect variations in the amounts of the hydrolytic products of the major maca storage compounds present in the tubers, which can in turn affect their nutraceutical properties.

In this work, the effects of the drying process on the levels of the reportedly bioactive macamides and of their putative metabolic precursors were investigated (Fig. 2) during traditional and industrial postharvest processing practices. We show that the amides are an intrinsic product of the drying process that involves the hydrolytic release and metabolic transformation of lipids and glucosinolates into their free fatty acid and benzylamine precursors.

2. Results and discussion

2.1. Amide, amine and free fatty acid content in dried maca

Macamides are bioactive secondary benzylalkylamides reported in *L. meyenii* hypocotyls (Ganzera et al., 2002; McCollom et al., 2005; Zhao et al., 2005; Muhammad et al., 2002). Although the material used for analysis in these publications consisted of dried hypocotyls or commercially available products obtained from them, it has been assumed that the macamides were present also in fresh hypocotyls as is the case with other species reported to contain alkamides (Martin-Tanguy et al., 1978; Greger, 1984; Molina-Torres et al., 1999; Clifford et al., 2002). In the study herein, HPLC analysis of freshly or of fresh freeze-dried maca showed very low or undetectable levels of the amides (Fig. 3a). For this reason, a comparative analysis was carried out of material obtained under the traditional open-air drying method with fresh freeze-dried samples. This analytical sequence (Fig. S2) was chosen to provide a simplified procedure for the determination of a number of expected hydrolysis products (free fatty acids, amines, isothiocyanates and other volatile organic compounds (VOCs)) along with the glucosinolate (**1**) and amide (**11**–**13**) levels. Table 1 shows a comparison of the levels of these metabolites in both preparations during our initial evaluation of International Potato Center (CIP, Lima) germplasm or of commercial samples. Fresh freeze-dried material consistently showed high levels of benzyl glucosinolate (BGl, **1**) but low, or undetectable, levels of free fatty acids (**9**, **10**), benzyl amine (BNH_2, **7**) and amides (**11**–**13**) (see Figs. S4 and S5 for LC profiles of BGl and BNH_2 in fresh and dried material). The traditionally dried material consistently showed levels of amides, amines and free fatty acids that were at least one order of magnitude higher, and BGl (**1**) levels that were less than one-third of those of fresh

Table 1
Secondary metabolites in dry maca hypocotyls obtained by either freeze drying of fresh material frozen in liquid nitrogen or by traditional open-air drying. Results are the average of seven independent experiments ($n = 7$). Samples were analyzed from the same harvested lots.

Metabolite	Fresh lyophilized maca	Traditionally dried maca
Macamides (**11**–**13**)	11.9 ± 1.9 μg g^{-1} dry wt	629.4 ± 97.4 μg g^{-1} dry wt
Free fatty acids (**9**, **10**)	47.2 ± 3.0 μg g^{-1} dry wt	600.0 ± 112.1 μg g^{-1} dry wt
Benzyl isothiocyanate (**2**)	475.0 ± 234.8 μg g^{-1} dry wt	21.5 ± 3.3 μg g^{-1} dry wt
Benzyl glucosinolate (**1**)	46.3 ± 4.7 mg g^{-1} dry wt	17.8 ± 2.5 mg g^{-1} dry wt
Benzylamine (**7**)	0.47 ± 0.07 mg g^{-1} dry wt	3.20 ± 0.40 mg g^{-1} dry wt

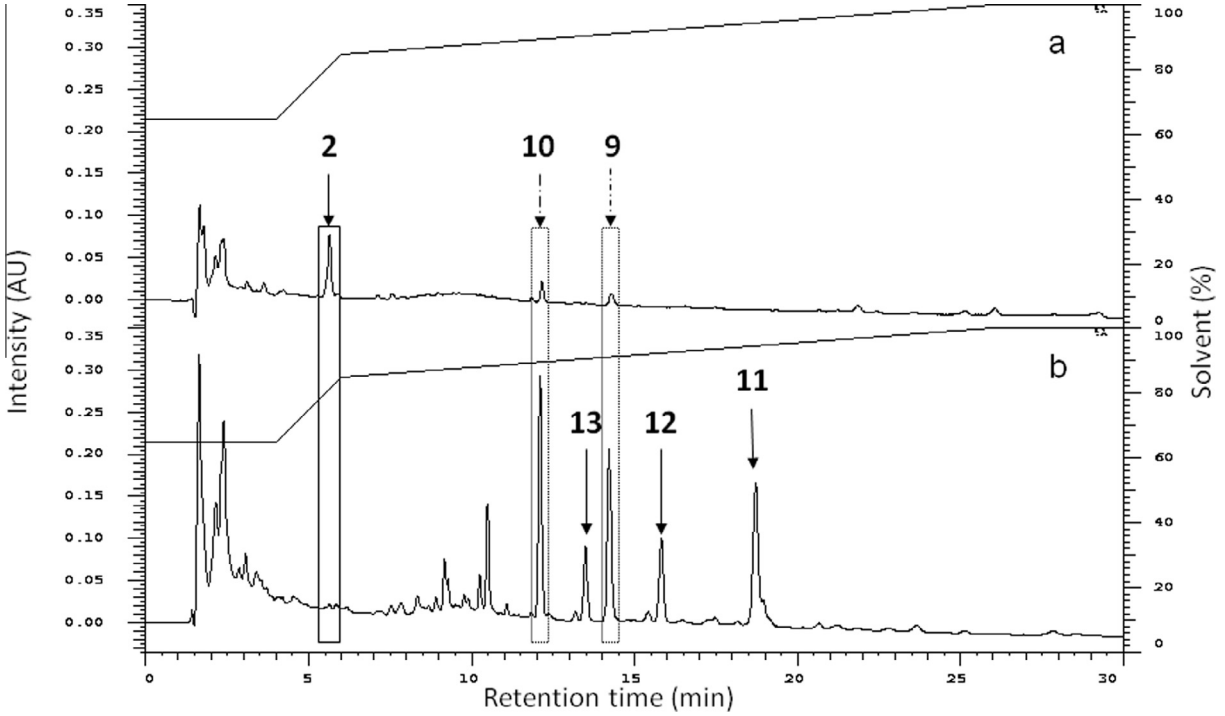

Fig. 3. Amide and fatty acid HPLC profiles of fresh and dry maca. MeOH extracts of fresh lyophylized (a) and open field dried (b) hypocotyls were analyzed by reversed phase liquid chromatography. The traditionally dried material has significantly greater relative peak size for the C16:0 (**11**), C18:2 (**12**) and C18:3 (**13**) macamides and for free linoleic (**9**) and linolenic (**10**) (in dotted line boxes). Benzyl isothiocyanate (**2**) is shown in a solid line box. Elution was monitored at 210 nm.

material. The higher levels of benzyl isothiocyanate (BITC, **2**) observed in fresh material were a product of the expected high myrosinase activity in tubers which act upon the glucosinolates as soon as the tissue was processed. The reactivity of the isothiocyanates very likely prevents a higher accumulation of these degradation products but their potential interconversion, enzymatic or otherwise, likely feeds a pool of less reactive intermediates that may work as precursors for the BNH_2 (**7**) and for the amides (**11**–**13**).

2.2. Product profiles during traditional open-field drying

In order to evaluate the appearance of amides in maca during drying, tissue samples were analyzed throughout the whole nine-week field-drying procedure in the Junín high-plateau (see Fig. S3 for environmental conditions during open field drying). For this, samples from four different harvest lots were shipped weekly to Lima frozen in liquid N_2. Given that maca is a heterogeneous crop with a variety of pheno/chemo-types present, representative samples of the phenotypes present in the particular lot were included. Another criterion was to standardize the size of the tubers in each lot at the start of the procedure to reduce variation in the drying rates. Assays were conducted for BGl (**1**), free linoleic (**9**) and linolenic acids (**10**), BITC (**2**) and BNH_2 (**7**), considering that all of these were potential direct or indirect biosynthetic sources, and the major macamides, 16:0 (**11**), 18:2 (**12**) and 18:3 (**13**).

Fig. 4 shows the changes in the levels of the selected products during the drying process. Water content in the tissues decreased over 9 weeks to a minimum value of 13%, which is the average content in dry maca. During this period, BGl (**1**) content showed a slight rise during the first two weeks of drying and then decreased continuously until enzyme inactivation at 13% water content. The initial rise in glucosinolate levels during drying has been also reported elsewhere (Yábar et al., 2011) and has to do with continued synthesis during the first week after harvest when the hypocotyls have not sustained enough damage by freeze–thaw cycles. As a consequence of tissue damage, BITC (**2**) accumulates as a major product of myrosinase activity. Initially, BITC (**2**) is present in quantifiable amounts, but as time passes, other myrosinase hydrolysis products, like benzylnitrile (BCN, **4**) or benzylisocyanate (BIOC, **3**) appear more prevalent in amount (Williams et al., 2009). In addition, there are a higher levels of BITC (**2**) hydrolysis or higher accumulation of oxidation products, like benzaldehyde (BCHO, **6**) and benzylalcohol (BOH, **5**). This is consistent with observations in our lab where the major detectable volatile product of fresh maca is BITC (**2**) while that of traditionally dried maca is BCN (**4**) (data not shown). Given the degree of metabolic processing of glucosinolates during the drying process, the observed rise in free BNH_2 (**7**) in the tissues could be explained as arising from protein hydrolysis and aromatic amino acid decarboxylation, or from BGl (**1**) hydrolysis and metabolism. Given the amounts involved in macamide formation, the latter pathway seems more likely. BNH_2 (**7**) has been reported as a product of BITC (**2**), BIOC (**3**) and BCN (**4**) hydrolysis (Olsen and Sørensen, 1980; Mewis et al., 2012). The rise in BNH_2 (**7**) correlates well with the evolution of both BGl (**1**) (-0.835, $P = 0.00514$) and BITC (**2**) (-0.920, $P < 0.005$) profiles (Table 2). Yábar and collaborators (2011) also describe a lower myrosinase activity during a field drying process after 90 days of drying (about 20–30%), but still having activity.

On the other hand, a significant rise in the concentration of unsaturated free fatty acids (FFAs, **9** and **10**) was also observed and is likely due to hydrolysis of reserve and membrane lipids. Correlating with the rise in FFAs (**9**, **10**) and BNH_2 (**7**) is an increase in macamide (MAC, **11**, **12** and **13**) content. There is a lag in the appearance of macamides compared to the rise in unsaturated FFAs (**9**, **10**) and BNH_2 (**7**) but all three profiles correlate well (Table 2). This correlation strongly suggests that the hydrolytic processes that accompany open-air drying are responsible for the formation of the precursors of macamides. It must be noted that macamide synthesis is still occurring until the end-point of the drying process at 13% tissue water content (Fig. 4).

2.3. Product profiles during oven drying

The product profiles from open-field drying trials showed a slow and steady buildup of MACs (**11**, **12**, **13**). Thus the question was addressed as to whether the traditional drying process, with its long exposure to temperature cycles, high irradiance, low

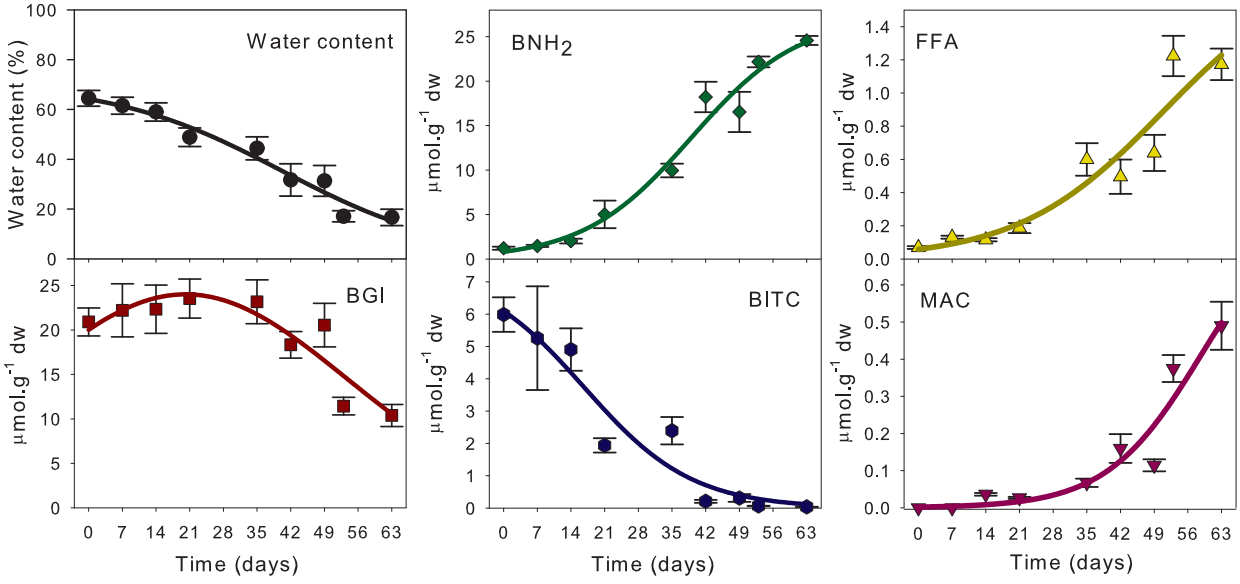

Fig. 4. Profiles of selected storage and secondary metabolites during traditional open-field drying of whole maca hypocotyls. Top left panel shows the decrease in tissue water content during the drying. Other panels show the levels of various secondary metabolites during the drying process: BGl (**1**): benzylglucosinolate, BITC (**2**): benzylisothiocyanate, BNH_2 (**7**): benzylamine, FFA: 18:2 (**9**) and 18:3 (**10**) free fatty acids and MAC: C16:0 (**11**), C18:2 (**12**) and C18:3 (**13**) macamides. Data shown corresponds to analyses performed in triplicate from four different lots of hypocotyls. Error bars represent standard error.

Table 2
Correlation analysis of metabolite profiles during the field drying process. Metabolites analyzed were, BGI (**1**): benzyl glucosinolate, BITC (**2**): benzyl isothiocyanate, BNH_2 (**7**): benzylamine, FFA (**9**, **10**): free linoleic and linolenic acid and MAC (**11**–**13**): sum of C16:0, C18:2 and C18:3 benzyl alkamides. Total sample number was 9 and corresponds to the time points in each one of the graphs in Fig. 4.

Compound	BGI (**1**)			BITC (**2**)			BNH_2 (**7**)			FFA (**9**, **10**)		
	r^a	P^b	N^c	*r*	*P*	*N*	*r*	*P*	*N*	*r*	*P*	*N*
BITC (**2**)	0.605	0.085	9									
BNH_2 (**7**)	−0.835	0.005	9	−0.920	<0.005	9						
FFA (**9**, **10**)	−0.874	<0.005	9	−0.805	0.009	9	0.934	<0.005	9			
MAC (**11**–**13**)	−0.965	<0.005	9	−0.721	0.028	9	0.898	<0.005	9	0.934	<0.005	9

[a] Correlation coefficient.
[b] *P* Value.
[c] Number of samples.

ambient humidity and tissue damage through repeated handling were essential conditions for amide accumulation. To evaluate this, the oven drying conditions were tested, these being used by maca flour exporters, and which involve shredding the hypocotyls and drying them at temperatures between 45 and 60 °C. The experiments were carried out with fresh maca which was shredded to 1 mm thick strips about 2 × 0.5 cm using a standard kitchen vegetable shredder. The resulting size was similar to that of strips obtained from large commercial shredders. The strips were placed to dry on standard glass petri dishes in a laboratory oven at 45 °C. Samples were taken at 0, 1, 2, 5, 10 and 24 h and analyzed as described in the previous section. The results (Fig. 5) show that, after shredding, the rise in free BNH_2 (**7**) amounts is fast, reaching a maximum after 5 h, with this remaining stable for the next 19 h. The rise in BNH_2 (**7**) is preceded by an extremely fast rise in BITC (**2**) concentrations, which then drop in a pattern that matches the rise in the free amine (**7**) levels. On the other hand, the rise in FFAs (**9**, **10**) and MACs (**11**–**13**) seems to follow a relatively constant rate for the first 10 h and then appears to taper off towards 24 h. The slow decline in the rates of their accumulation correlate well with the drop in the moisture content of the tissues, i.e., by 10 h, they have reached 5% moisture level, with a concomitant slowing of most enzymatic processes. The extremely fast rise and decline in BITC (**2**) levels and the fast rise in BNH_2 (**7**) is consistent with a decline of about 50% in BGl (**1**) content in the tissues. The correlation in the profiles (Table 3) of glucosinolate and lipid breakdown and rise in metabolite and amide levels is good. Correlation coefficients (*r*) of the BGl (**1**) and FFA (**9**, **10**) profiles to that of MAC (**11**–**13**) accumulation showed good values of −0.933 and 0.974, respectively.

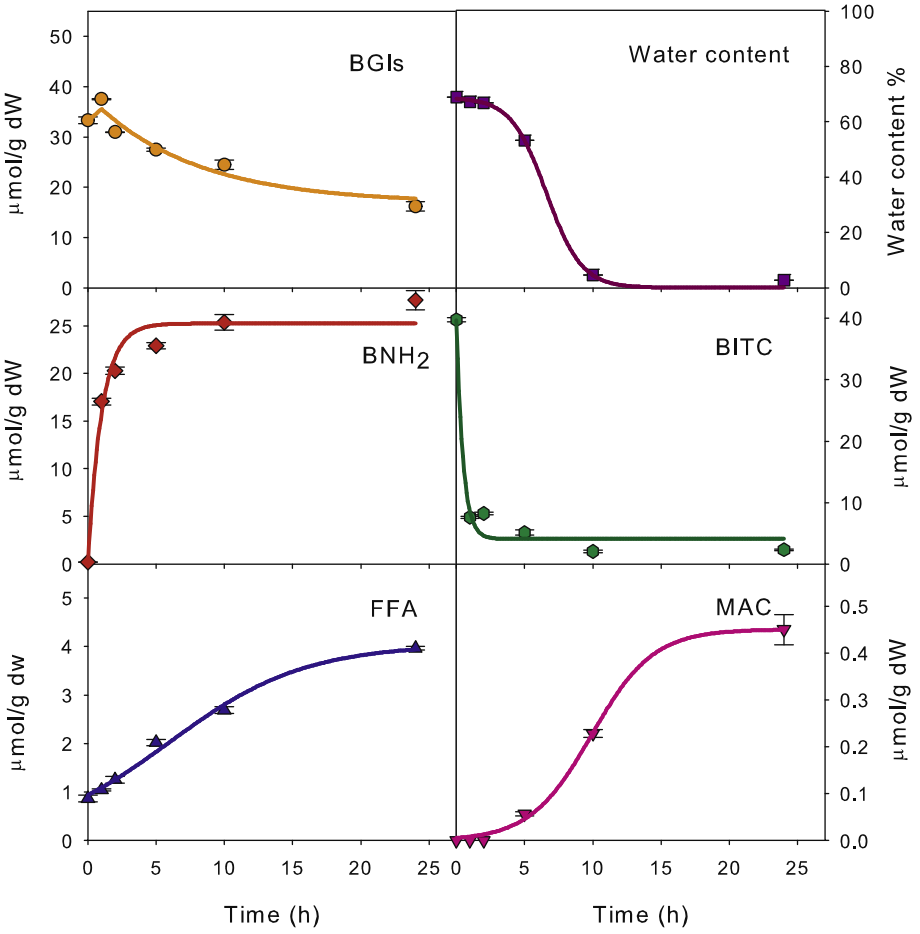

Fig. 5. Profiles of selected metabolites during oven-drying of shredded maca hypocotyls at 45 °C. For abbreviations see the legend of Fig. 4. Experiments were performed in triplicate with one lot of maca hypocotyls. Error bars represent standard error.

Table 3
Correlation analysis of maca postharvest metabolite profiles during oven drying. Pearson correlation analysis of data obtained from oven drying experiments. The first five columns and rows correspond to volatiles analyzed by headspace SPME GC–MS. The following four were analyzed by HPLC. Sample size was 6 or 7 as shown and corresponds to data shown in Figs. 5 and 6. VOCs analyzed were BCN (**4**): benzyl nitrile, BIOC (**3**): benzyl isocyanate, BITC (**2**): benzyl isothiocyanate, BOH (**5**): benzyl alcohol. For abbreviations of other metabolites see Table 2. FFA refers exclusively to the sum of linoleic (**9**) and linolenic (**10**) acids, and MAC to MAC 16 (**11**), MAC 18:2 (**12**) and MAC 18:3 (**13**).

		BCN (**4**)	BIOC (**3**)	BITC[d] (**2**)	BOH (**5**)	BNH_2 (**7**)	BGl (**1**)	BITC[e] (**2**)	FFA (**9**, **10**)	MAC (**11**–**13**)
BCHO (**6**)	r[a]	0.9090	0.2920	0.2890	0.6520	0.7330	−0.3870	−0.7340	0.4500	0.3200
	P[b]	0.0045	0.5260	0.5300	0.1130	0.0975	0.4490	0.0968	0.3700	0.5360
	N[c]	7	7	7	7	6	6	6	6	6
BCN (**4**)	r		0.4610	0.4770	0.7310	0.4970	−0.6790	−0.4020	0.7660	0.8120
	P		0.2980	0.2790	0.0620	0.3160	0.1380	0.4290	0.0756	0.0495
	N		7	7	7	6	6	6	6	6
BIOC (**3**)	r			0.9920	−0.2120	−0.9520	0.7250	0.8890	−0.7690	−0.6160
	P			<0.001	0.6490	0.0034	0.1030	0.0179	0.0741	0.1930
	N			7	7	6	6	6	6	6
BITC[d] (**2**)	r				−0.1880	−0.9130	0.7240	0.8340	−0.7270	−0.5620
	P				0.6870	0.0111	0.1040	0.0390	0.1020	0.2450
	N				7	6	6	6	6	6
BOH (**5**)	r					0.7660	−0.9350	−0.6270	0.9820	0.9570
	P					0.0757	0.0062	0.1820	<0.001	0.0027
	N					6	6	6	6	6
BNH_2 (**7**)	r						−0.6550	−0.9720	0.7430	0.6230
	P						0.1580	0.0011	0.0905	0.1860
	N						6	6	6	6
BGl (**1**)	r							0.4650	−0.9650	−0.9330
	P							0.3520	0.0018	0.0066
	N							6	6	6
BITC[e] (**2**)	r								−0.5910	−0.4680
	P								0.2160	0.3490
	N								6	6
FFA (**9**, **10**)	r									0.9740
	P									<0.001
	N									6

[a] Correlation coefficient.
[b] *P* Value.
[c] Number of samples.
[d] BITC in headspace by SPME analysis.
[e] BITC analyzed by HPLC together with macamides.

2.4. VOC profiles in oven-dried hypocotyls

Oven drying, as opposed to field drying, provided the opportunity to follow the time course of volatile organic byproducts (VOCs) of glucosinolate hydrolysis. To evaluate this, freshly shredded maca strips were placed inside glass chambers in an oven at 45 °C (Fig. S7) and headspace solid phase microextraction (HS-SPME) analysis of the volatiles present in the chamber was performed at various times during the drying process. SPME fibers with three stationary phases (Supelco) were chosen as these have proven useful with the range of polarities present in other plant volatile emissions evaluated in our laboratories. Exposure of the fibers was made over a 30 min period which included also chamber saturation time. Exposure and chamber saturation took place simultaneously. This was done to allow quicker sampling for the initial times and to avoid carry-over of VOCs by accumulation in the chamber. The chamber was fully vented between measurements. This did not allow a clear quantitative measurement but, as the results show, it did provide a representative profile of the sequence of VOC emissions. The results (Fig. 6) show a very sharp rise in BITC (**2**) levels and its corresponding isocyanate (BIOC, **3**) during the first few minutes, right after shredding and heating of the tissues. These levels drop quickly over the following 5 h. The next set of VOCs, however, starts rising after BITC (**2**) decline sets in. Benzaldehyde (BCHO, **6**) and benzylalcohol (BOH, **5**) peak between 4 and 8 h and benzylnitrile (BCN, **4**) has a very broad peak at 8 h after shredding. The general lipid breakdown indicator, hexanal (**8**), rises slowly and reaches a plateau at 8 h which then holds for the whole measurement period. Although assay conditions differ, if the results from HPLC (Fig. 5) and GC (Fig. 6) experiments with oven-dried shredded maca are compared, it can be seen that the profiles for BNH_2 (**7**), BCHO (**6**) and BOH (**5**) peak around similar times and exhibit a good correlation (Table 3). The only difference between BNH_2 (**7**) levels, and the other VOCs is that the amine accumulates over time, but given the way in which headspace determinations were made, and the nature of SPME VOC capture itself, it is difficult to conclude whether these compounds do not have a higher concentration in the macerated tissues. What can be concluded reliably is that the amine (**7**), aldehyde (**6**) and alcohol (**5**) all seem be generated in the same sequence. On the other hand, the lipid breakdown indicator hexanal also follows the general pattern for the release of free fatty acids and the emission of this VOC indicates that a significant degree of fatty acid degradation (very likely including peroxidation and hydroxylation) is taking place throughout the whole time period where one observes amide accumulation.

2.5. Post-harvest metabolism

Postharvest metabolism in maca undergoing open-field traditional drying is determined by a combination of mechanical damage, freeze–thaw cycles and dehydration of the tissues in an environment with high irradiance, low humidity and extremes of temperature (Fig. S4). These manipulations, as with other postharvest processing procedures that involve maceration, result in progressive tissue damage with the release of hydrolytic enzymes. The action of lipases on reserve and membrane lipids accounts for the rise in FFAs (**9**, **10**) which can also undergo hydroxylation and peroxidation reactions as seen from the release of hexanal in the samples. No detailed analysis of FFA modifications during the drying process was carried out. The study focused on linoleic (**9**) and linolenic (**10**) FFA levels which can be easily monitored by HPLC at 210 nm, with structure confirmation by ESI-MS.

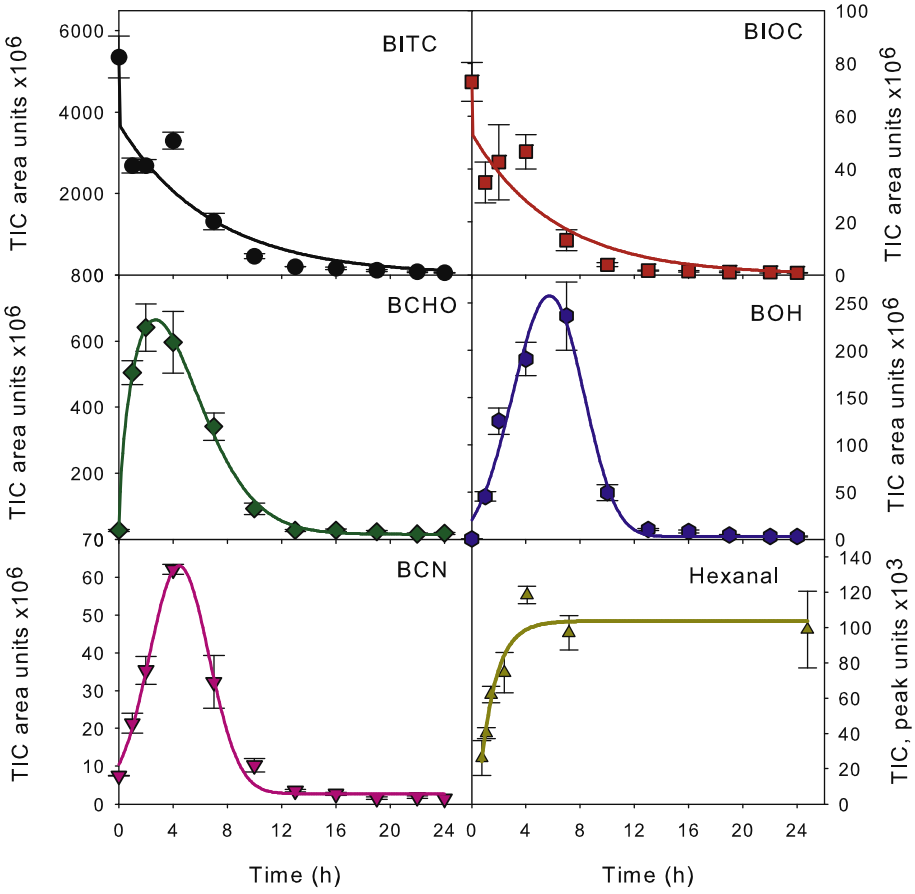

Fig. 6. Profiles of volatile metabolites (VOCs) obtained by headspace solid phase microextraction (HS-SPME) and GC–MS analysis from shredded maca hypocotyls during oven-drying at 45 °C. From top left to bottom right panels, BITC (**2**): benzyl isothiocyanate, BIOC (**3**): benzyl isocyanate, BCHO (**6**): benzaldehyde, BOH (**5**): benzyl alcohol, BCN (**4**): benzyl nitrile and hexanal (**8**). Values shown are peak area averages obtained from total ion GC–MS chromatograms (TIC) in three independent samplings, error bars show standard error.

On the other hand, the rise in free BNH_2 (**7**) levels cannot be accounted for by protein hydrolysis, as its tissue levels on a molar basis are an order of magnitude higher than those of free phenylalanine and they show a relatively flat profile throughout the drying process (see Fig. S6). More likely, BNH_2 (**7**) arises from glucosinolate metabolism given that BGl (**1**) is the main compound accumulating in maca. The results presented here show matching sequences for the appearance of BGl (**1**) degradation products in both open field and in oven dried material and can be seen in both liquid and gas chromatographic analyses. Metabolic conversion between BITC (**2**), BCHO (**6**), BOH (**5**) and BNH_2 (**7**) may be mediated by endogenous enzymes or, for the case of the long-term drying process in the field, by microbial enzymes present during tissue maceration. Conversion of BITC (**2**) into BNH_2 (**7**) in papaya pulp has been reported (Tang et al., 1972) as being carried out by bacteria from the genus *Enterobacter*. Also, there is a number of later reports of the conversion of BITC (**2**), BIOC (**3**) and BCN (**4**) into BNH_2 (**7**) in plant and mammalian tissues and by bacteria (Goosen et al., 2001; Cheng et al., 2004; Bones and Rossiter, 2006; Gimsing et al., 2007; Bednarek et al., 2009; Williams et al., 2009).

Our work has not, however, dealt with the appearance of macaenes (Zhao et al., 2005) which are oxidized derivatives of 18:2 and 18:3 fatty acids and also appear as part of the macamide structure; their processing is though very likely similar to that of N-alkyl ethanolamides (NAEs) in plants, where the involvement of lipoxygenases has been well documented for *Arabidopsis thaliana* (Shrestha et al., 2002). Desiccation and tissue damage are two processes which are reported to trigger amide synthesis in plant tissues (Greger, 1984; Sendker and Nahrstedt, 2008). During the drying procedure, it is therefore likely that BNH_2 (**7**) and free fatty acids (**9**, **10**) come into contact with an enzymatic amide generating system. The synthesis of macamides could potentially take place by the action of a fatty acid amide hydrolase (FAAH) working in reverse (Fig. 7). This has been shown for the synthesis of anandamide, the endocannabinoid neurotransmitter in mammalian tissues (Devane and Axelrod, 1994; Kruszka and Gross, 1994; Ueda et al., 1995; Högestätt et al., 2005) and can possibly occur in plants as in the case of the synthesis of the small amounts of amides during the maceration of cocoa beans.

2.6. Conclusions

Many traditional crop postharvest processes involve tissue damage that alters chemical profiles and generates bioactive metabolites absent from freshly harvested material. In this work, it was found that the traditional open-field drying of maca (*L. meyenii* Walp.) hypocotyls in the Andes, or industrial flaking and oven drying, results in hydrolytic processing of lipids and glucosinolates and the release of significant amounts of free unsaturated fatty acids (**9**, **10**) and benzylamine (**7**), both of which are precursors of, and whose accumulation correlates well with, the synthesis of macamides (**11**, **12**, **13**). The series of reactions involved (Fig. 7) has previously been shown in the literature in other systems to take place through the action of endogenous or microbial enzymes or through nonenzymatic processes. The

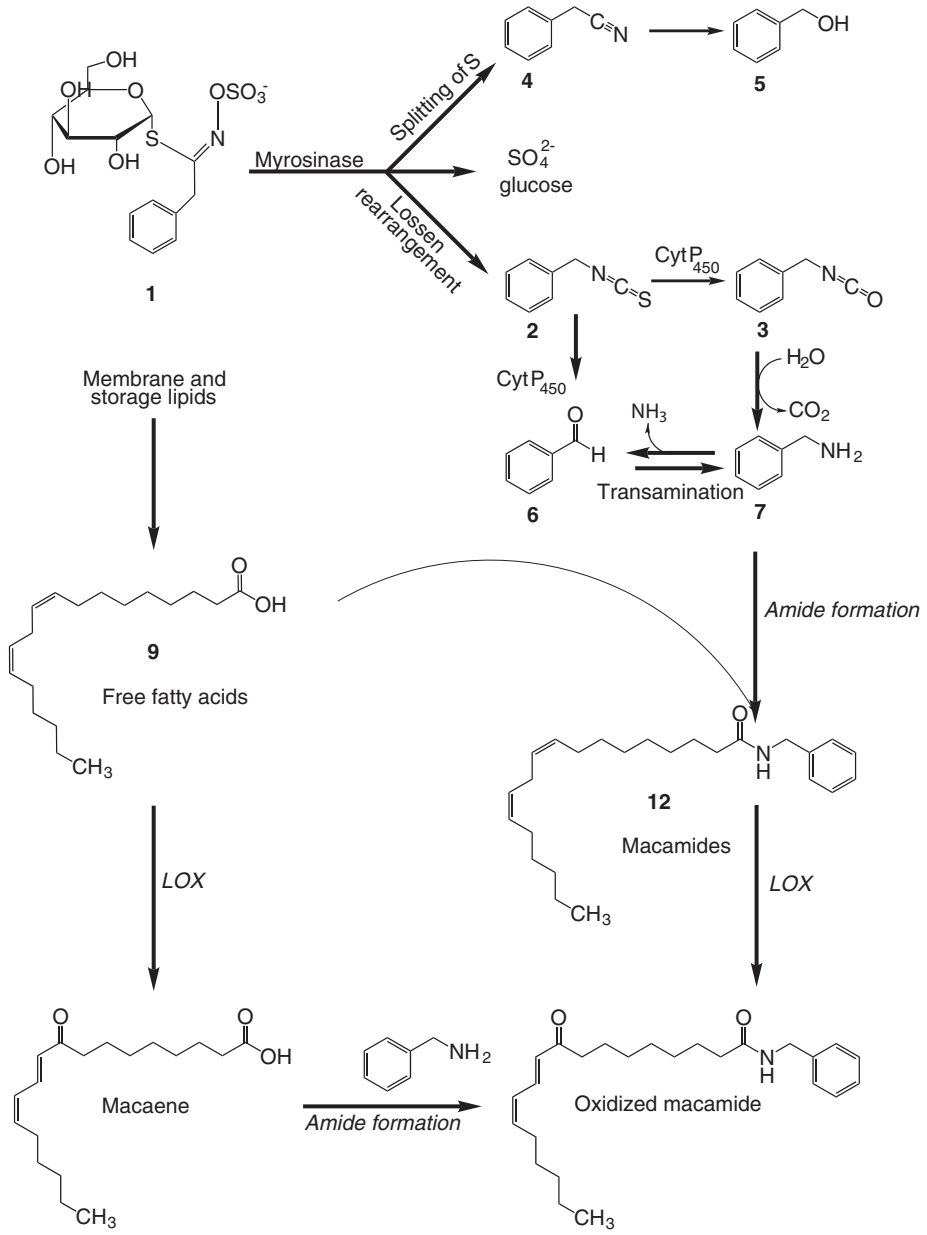

Fig. 7. A proposed biosynthetic scheme for macamides (**12**) in *L. meyenii* drying tissues. It is assumed that the main substrates are free fatty acids (**9**) and benzylamine (**7**), arising from hydrolysis of storage triacylglycerols and membrane lipids and of glucosinolate (**1**) hydrolysis to benzyl isothiocyanate (BITC, **2**) and to benzylamine (BNH_2, **7**).

proposed reverse action of an endogenous fatty acid amide hydrolase (FAAH) for the synthesis of amides has also been the subject of discussion in the literature. This opens the question whether other glucosinolate-containing plants can generate amides when undergoing disruptive tissue drying. Given that macamides play a stimulatory role on the central nervous system through inhibition of mammalian FAAH and the associated endocannabinoid receptor system, an understanding of the as yet unrecognized relevance of the post-harvest metabolism of this crop should help develop products with improved chemical profiles and biological activity.

3. Experimental

3.1. General procedures and instrumentation

Structural characterization of synthetic amides was performed using a Bruker 300 UltraShield NMR (Karlsruhe, Germany), a Perkin Elmer series 1600 (Waltham, MA) FT-IR spectrometer and a ThermoSpectronic Genesys 6 (Rochester, NY) UV/Vis spectrophotometer. Data loggers for environmental variables during field drying studies were OM-62 units from Omega Engineering (Stamford, CN) and a XR5 SE system from Pace Scientific (Mooresville, NC) equipped with a TRH-100 temperature and humidity sensor and a SRS-100 solar radiation meter.

Statistical treatment and nonlinear regression analyses were performed using SigmaPlot 11 for Windows (Systat Software).

3.2. Chemicals

Benzylglucosinolate (**1**) was obtained from Calbiochem/Merck Biosciences (San Diego, CA). Benzylisothiocyanate (**2**), benzylnitrile (**4**), benzyl alcohol (**5**), benzaldehyde (**6**), benzylisocyanate (**2**), fatty acids (**9**, **10**) and reagents for amide synthesis were obtained from Sigma–Aldrich (St. Louis, MO). Hexanal was from

Accustandard (New Haven, CT). All other solvents and reagents were analytical or HPLC grade.

3.3. Synthesis of macamide standards

The benzylamides of palmitic, linoleic and linolenic acids were synthesized for use as standards for quantification in the work. For this, the general method of Kataoka and coworkers (1996) was adapted as follows: fatty acid (1 mmol), 4-pyrrolidinyl-pyridine catalyst (0.5 mmol) and benzylamine (1.5 mmol) were mixed in CH_2Cl_2 (10 mL) under constant stirring at 0 °C, after which N,N-dicyclohexylcarbodiimide (1.0 mmol) dissolved in CH_2Cl_2 (5 mL), was slowly added to the mixture (Montalbetti and Falque, 2005). Reaction progress was monitored by TLC in 10 cm Si 60 F254 plates (Merck, Darmstadt, Germany) using CH_2Cl_2 as mobile phase.

The reaction mix was filtered through Whatman GF-A filters, with the filtrate extracted with 10% aq. NaOH (×3); this procedure was then repeated using 10% HCl. The extracted organic phase was dried (anhydrous Na_2SO_4), filtered, and diluted with half a volume of hexane. The mixture was subjected to silica-gel 60 column chromatography (4 g, 10 × 1 cm column) and conditioned with CH_2Cl_2–hexane (10 mL, 2:1). After loading the sample, the column was washed with of CH_2Cl_2–hexane (4 mL, 2:1). Impurities were eluted with CH_2Cl_2–hexane (8 mL, 3:1) and the amide fraction with pure CH_2Cl_2 followed by CH_2Cl_2–EtOAc (8:1). The two eluates were combined, evaporated to dryness and stored at −20 °C under N_2 gas. The synthetic amides were characterized by NMR, IR, LC–MS and GC–MS (see Supplementary Information for spectroscopic data of the synthetic amides).

3.4. Plant material

Four independently harvested lots of maca, from the fields of four different producers associated to Ecoandino S.A.C. (Lima, Peru) were placed to dry in the open air on a field located at 4128 m altitude, 2 km north of the city of Junin (UTM 18 L 391976m E, 8768061m S) in central Peru between June and August of 2011 and 2012. All lots were dried under the same conditions and were exposed to the air during the day and covered at night with plastic tarpaulin to avoid frost.

On a weekly basis, random samples of 10 hypocotyls (20–100 g total weight) between 0.5 and 4 cm in diameter were taken from each of the 4 lots and placed directly in metal baskets in 10 L CX-100 Taylor-Wharton containers filled with liquid N_2. The containers were shipped to our laboratory and remained in liquid N_2 until analyzed. The samples contained an assortment of the known maca phenotypes (black, purple, yellow and red).

For oven drying tests in the laboratory, fresh yellow maca hypocotyls were obtained from a local market in Lima, Peru, with a uniform size, approximately 3 cm in diameter. These were washed with distilled H_2O, pat-dried with filter paper and shredded. Then, they were placed in Petri dishes, which were placed in an oven at 45 °C. Samples were taken at 0, 1, 2, 5, 10 and 24 h for HPLC analysis and at 0, 1, 2, 4, 7, 10, 13, 16, 19, 22 and 24 h for VOC analysis by headspace GC–MS.

3.5. Extraction of plant material

Maca hypocotyls, frozen in liquid N_2 were ground in an IKA A11 (Staufen, Germany) analytical mill with portions (1 g) placed directly in polypropylene centrifuge bottles (50 mL) containing H_2O–MeOH (10 mL, 30:70, v/v) preheated to 70 °C. The bottles were flushed with N_2 gas, closed and placed in a shaking water bath at 70 °C for 1 h. They were then centrifuged at 10,000×*g* for 15 min. The supernatant was vacuum filtered through Whatman GF/A filters and the filtrate collected in glass culture tubes (50 mL) with Teflon-lined caps. The pellet was re-extracted for 15 min (×2) and the filtered supernatants were pooled. Samples were stored under N_2 at −20 °C until analyzed. The same procedure was used for shredded maca in oven-drying experiments.

3.6. Analysis of amides, free fatty acids and isothiocyanates

Macamides (**11**–**13**), free unsaturated fatty acids (**9**–**10**) and benzyl isothiocyanate **2** were analyzed as a modification of the methods reported by McCollom and coworkers (2005) and Ganzera and coworkers (2002). Samples were in H_2O–MeOH (50:50, v/v) with 7 mL loaded onto a Merck Lichrolut RP-18 (500 mg) reversed phase SPE column. The columns were washed with H_2O–MeOH (5 mL, 50:50, v/v) and eluted with MeOH (2 mL). HPLC analysis of the eluate was carried out in a Merck-Hitachi LaChrom D-7000 HPLC quaternary pump system with column oven coupled to a L-7450 A diode array detector. A Merck LiChrospher 100 RP-18, 250 mm × 4.6 mm i.d. (5 μm) column was used for separations. The solvent program is shown in Table S1 in Supplementary Information. The column oven was set to 40 °C, flow at 1 mL min^{-1}, and the injection volume was 20 μL. Elution was monitored at 210 nm. The results present total macamides as a sum of compounds **11**–**13**. Free fatty acids (FFA) are the sum of compounds **9** and **10**.

3.7. LC–MS analysis of macamides

LC–MS analyses were carried out on an Agilent 1200 binary pump HPLC system coupled to a 1260 Agilent Infinity diode array detector connected in series with a Bruker Esquire 6000 ion trap ESI-MS. The column used was a Merck LiChrospher 100 RP-18, 250 mm × 4.6 mm i.d. (5 μm). Runs were made at 40 °C and a flow of 1 mL min^{-1}. Solvent A consisted of MeOH–H_2O (95:5, v/v) and B of CH_3CN. Both solvents contained 0.005% HCOOH. The elution gradient was modified due to it being a binary gradient instrument, unlike in the previous section. An initial 4 min at 65% B followed by a 2 min gradient from 65% to 85% B, 20 min from 85% to 100% B and a final 10 min at 100% B. ESI-MS parameters were the following: 70 psi nebulizer pressure, 12 L min^{-1} N_2 flow, 365 °C nebulizer temperature; high voltage capillary at −4000 V, capillary exit at 116.9 V, skimmer 40.0 V, trap drive 41.0 and scan range (*m*/*z*) 125–700.

3.8. Analysis of benzylamine and free amino acids

Amines were analyzed by derivatization with *o*-phthaldialdehyde (OPA) followed by reversed phase HPLC. Each pooled MeOH extract (200 μL) was diluted with 0.4 M Na borate buffer (250 μL, pH 9.5) before adding OPA reagent (50 μL). The reagent contained OPA (27 mg) in EtOH (0.5 mL) diluted with sodium borate buffer (5 mL) and 2-mercaptoethanol (25 μL). The mix was vortexed for 1 min, left at room temperature for 3 min and 20 μL were injected for HPLC analysis.

HPLC instrumentation was the same as for macamide analyses. The column used was a Merck LiChrospher 100 RP-18, 125 mm × 4.6 mm i.d. (5 μm). Oven temperature was kept at 30 °C and detection was performed at 340 nm. The solvent system consisted of MeOH (solvent A) and 20 mM NaOAc buffer, pH 6.0 (solvent B). The program consisted of a 9 min gradient from 40% to 100% A and 4 min at 100% A (see Fig. S5).

The same procedure was used for amino acid analysis except that the HPLC gradient was changed to begin at 15% A, held for 5 min, then from 15% to 25% A in 5 min and held at 25% A for 2 min. After that, taken to 50% A in 12 min, then to 70% A in 9 min, and finally to 100% A in 9 min and held at 100% A for 6 min.

3.9. Analysis of glucosinolates

Benzylglucosinolate (**1**) was analyzed as its desulfated counterpart with slight modification from the literature (Brown et al., 2003; Møldrup et al., 2011) Aliquots of the pooled hypocotyl extracts were diluted in H_2O–MeOH (50:50, v/v) with 1.5 mL applied onto conditioned Agilent Bond Elut-SAX SPE columns (500 mg). The latter were washed sequentially with MeOH–H_2O (5 mL, 70:30, v/v) and then conditioned with 0.02 M MES buffer (1 mL, pH 5.2) after which 150 μL of sulfatase (H-1, *Helix pomatia*, Sigma, St. Louis, MO) was added and the columns were incubated overnight at room temperature. The following day desulfoglucosinolates were eluted with MeOH–H_2O (800 μL, 70:30, v/v) and H_2O (800 μL). Both eluates were pooled in 2 mL autosampler vials and used directly for HPLC analysis.

HPLC analysis was performed using a Merck LiChrospher 100 RP-18, 250 mm × 4.6 mm i.d. (5 μm) column, oven temperature 30 °C and a 1 mL min^{-1} flow rate. Samples (20 μL) were injected and elution was monitored at 229 nm. The gradient program used CH_3CN (solvent A) and H_2O (solvent B) and was as follows: 6 min from 2% A to 5% A, 2 min 5% A to 7% A, 10 min 7% A to 21% A, 5 min 21% A to 29% A, 2 min 29% A to 100% A and 2 min at 100% A (Brown et al., 2003).

LC–MS analysis used the same column and gradient described in the previous paragraph. ESI-MS parameters were configured as follows: nebulizer pressure 65.0 psi, 12 L min^{-1} N_2 flow, nebulizer temperature 365 °C, high voltage capillary −4000 V, capillary exit 113.5 V, skimmer 40.0 V, trap drive 37.8, scan range (m/z) 100–700 (Matthäus and Luftmann, 2000). For the aim of this paper only BGl (**1**), as the major glucosinolate, was identified and quantified.

3.10. VOC analysis

Headspace solid phase micro-extraction (HS-SPME) of volatiles was performed using fused silica fibers coated with a 30 μm layer of DVB/carboxen/PDMS (divinylbenzene/carboxen/polydimethylsiloxane) (Supelco, Bellefonte, PA). The fibers were conditioned following the manufacturer's instructions by exposure in the GC injection port at 270 °C for 30 min.

For the analysis, of shredded maca (∼10 g) were placed in a closed cylindrical glass chamber (13 × 11 cm) with a lid with three ports with injection septa. The chamber was placed in an oven at 45 °C. SPME fibers were introduced through the septa to expose them to the headspace within (see Fig. S7). SPME fibers were exposed to the headspace for 30 min. After exposure, the fibers were inserted into the injection port of the GC for analysis. GC–MS analysis was performed using an Agilent 7890 GC coupled to a 5977 quadrupole MSD. The GC was equipped with a DB-5 ms column (30 m × 0.25 mm, 0.25 μm film thickness). Injector temperature was set at 250 °C; split was 5:1, and helium carrier gas flow rate was 0.9 mL min^{-1}. The oven temperature was initially held at 45 °C for 4 min, and then raised to 280 °C at 12 °C min^{-1} and finally held for 3 min at 280 °C. Electron ionization was set at 70 eV. Identification of the compounds was performed by the use of pure standards, retention indices and by mass spectra. Mass spectral analysis used AMDIS 32 (version 2.69, NIST), the NIST Mass Spectral Search Program and NIST 2011 mass spectral library.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytochem.2015.02.030.

References

Bednarek, P., Pislewska-Bednarek, M., Svatos, A., Schneider, B., Doubsky, J., Mansurova, M., Humphry, M., Consonni, C., Panstruga, R., Sanchez-Vallet, A., Molina, A., Schulze-Lefert, P., 2009. A glucosinolate metabolism pathway in living plant cells mediates broad-spectrum antifungal defense. Science 323, 101–106.

Bones, A.M., Rossiter, J.T., 2006. The enzymic and chemically induced decomposition of glucosinolates. Phytochemistry 67, 1053–1067.

Brown, P.D., Tokuhisa, J.G., Reichelt, M., Gershenzon, J., 2003. Variation of glucosinolate accumulation among different organs and developmental stages of *Arabidopsis thaliana*. Phytochemistry 62, 471–481.

Canales, M., Aguilar, J., Prada, A., Marcelo, A., Huamán, C., Carbajal, L., 2000. Nutritional evaluation of *Lepidium meyenii* (Maca) in albino mice and their descendants. Arch. Latinoam. Nutr. 50, 126–133.

Cheng, D.L., Hashimoto, K., Uda, Y., 2004. In vitro digestion of sinigrin and glucotropaeolin by single strain of *Bifidobacterium* and identification of the digestive products. Food Chem. Toxicol. 42, 351–357.

Clément, C., Diaz, D., Manrique, I., Avula, B., Khan, I.A., Ponce Aguirre, D.D., Kunz, C., Mayer, A.C., Kreuzer, M., 2010. Secondary metabolites in maca as affected by hypocotyl color, cultivation history, and site. Agron. J. 102, 431–439.

Clifford, L.J., Nair, M.G., Rana, J., Dewitt, D.L., 2002. Bioactivity of alkamides isolated from *Echinacea purpurea* (L.) Moench. Phytomedicine 9, 249–253.

Devane, W.A., Axelrod, J., 1994. Enzymatic synthesis of anandamide, an endogenous ligand for the cannabinoid, by brain membranes. Proc. Natl. Acad. Sci. USA 91, 6698–6701.

Dini, A., Migliuolo, G., Rastrelli, L., Saturnino, P., Schettino, O., 1994. Chemical composition of *Lepidium meyenii*. Food Chem. 49, 347–349.

Ganzera, M., Zhao, J., Muhammad, I., Khan, I.A., 2002. Chemical profiling and standardization of *Lepidium meyenii* (maca) by reversed phase high performance liquid chromatography. Chem. Pharm. Bull. (Tokyo) 50, 988–991.

Gimsing, A.L., Poulsen, J.L., Pedersen, H.L., Hansen, H.C., 2007. Formation and degradation kinetics of the biofumigant benzyl isothiocyanate in soil. Environ. Sci. Technol. 41, 4271–4276.

Gonzales, G.F., Miranda, S., Nieto, J., Fernandez, G., Yucra, S., Rubio, J., Yi, P., Gasco, M., 2005. Red maca (*Lepidium meyenii*) reduce prostate size in rats. Reprod. Biol. Endocrinol. 3, 1–16.

Gonzales, G.F., Nieto, J., Rubio, J., Gasco, M., 2006. Effect of black maca (*Lepidium meyenii)* on one spermatogenic cycle in rats. Andrologia 38, 166–172.

Goosen, T.C., Mills, D.E., Hollenberg, P.F., 2001. Effects of benzyl isothiocyanate on rat and human cytochromes P450: identification of metabolites formed by P450 2B1. J. Pharmacol. Exp. Ther. 296, 198–206.

Greger, H., 1984. Alkamides: structural relationships, distribution and biological activity. Planta Med. 50, 366–375.

Högestätt, E.D., Jönsson, B.A., Ermund, A., Andersson, D.A., Björk, H., Alexander, J.P., Cravatt, B.F., Basbaum, A.I., Zygmunt, P.M., 2005. Conversion of acetaminophen to the bioactive N-acylphenolamine AM404 via fatty acid amide hydrolase-dependent arachidonic acid conjugation in the nervous system. J. Biol. Chem. 280, 31405–31412.

Kataoka, K., Shiota, T., Takeyasu, T., Minoshima, T., Watanabe, K., Tanaka, H., Mochizuki, T., Taneda, K., Ota, M., Tanabe, H., Yamaguchi, H., 1996. Potent Inhibitors of acyl-CoA:cholesterol acyltransferase. 2. Structure-activity relationships of novel N-(2,2-dimethyl-2,3-dihydrobenzofuran-7-yl)amides. J. Med. Chem. 39, 1262–1270.

Kruszka, K.K., Gross, R.W., 1994. The ATP- and CoA-independent synthesis of arachidonoylethanolamide. A novel mechanism underlying the synthesis of the endogenous ligand of the cannabinoid receptor. J. Biol. Chem. 269, 14346–14348.

Li, G., Ammerman, U., Quiroz, C.F., 2001. Glucosinolate contents in maca (*Lepidium peruvianum*, Chacón) seeds, sprouts, mature plants and several derived commercial products. Econ. Bot. 55, 255–262.

Martin-Tanguy, J., Cabanne, F., Perdrizet, E., Martin, C., 1978. The distribution of hydroxycinnamic acid amides in flowering plants. Phytochemistry 17, 1927–1928.

Matthäus, B., Luftmann, H., 2000. Glucosinolates in members of the family Brassicaceae: separation and identification by LC/ESI-MS-MS. J. Agric. Food Chem. 48, 2234–2239.

McCollom, M.M., Villinski, J.R., McPhail, K.L., Craker, L.E., Gafner, S., 2005. Analysis of macamides in samples of maca (*Lepidium meyenii*) by HPLC-UV-MS/MS. Phytochem. Anal. 16, 463–469.

Mewis, I., Schreiner, M., Nguyen, C.N., Krumbein, A., Ulrichs, C., Lohse, M., Zrenner, R., 2012. UV-B irradiation changes specifically the secondary metabolite profile in broccoli sprouts: induced signaling overlaps with defense response to biotic stressors. Plant Cell Physiol. 53, 1546–1560.

Møldrup, M.E., Geu-Flores, F., Olsen, C.E., Halkier, B.A., 2011. Modulation of sulfur metabolism enables efficient glucosinolate engineering. BMC Biotechnol. 11, 1–8.

Molina-Torres, J., Garćıa-Chávez, A., Ramírez-Chávez, E., 1999. Antimicrobial properties of alkamides present in flavouring plants traditionally used in Mesoamerica: affinin and capsaicin. J. Ethnopharmacol. 64, 241–248.

Montalbetti, C.A.G.N., Falque, V., 2005. Amide bond formation and peptide coupling. Tetrahedron 61, 10827–10852.

Muhammad, I., Zhao, J., Dunbar, C., Khan, I.A., 2002. Constituents of *Lepidium meyenii* "Maca". Phytochemistry 59, 105–110.

National Research Council (USA). Advisory Committee on Technology Innovation, 1989. Maca. In Lost crops of the Incas: little-known plants of the Andes with promise for worldwide cultivation. National Academy Press, Washington D.C., pp. 57–65.

Olsen, O., Sørensen, H., 1980. Glucosinolates and amines in *Reseda media*. Phytochemistry 19, 1783–1787.

Piacente, S., Carbone, V., Plaza, A., Zampelli, A., Pizza, C., 2002. Investigation of tuber constituents of maca (*Lepidium meyenii* Walp.). J. Agric. Food Chem. 50, 5621–5625.

Pino-Figueroa, A., Vu, H., Kelley, C.J., Maher, T.J., 2011. Mechanism of action of *Lepidium meyenii* (Maca): an explanation for its neuroprotective activity. Am. J. Neuroprot. Neuroregen. 3, 87–92.

Rubio, J., Dang, H., Gong, M., Liu, X., Chen, S., Gonzales, G.F., 2007. Aqueous and hydroalcoholic extracts of black maca (*Lepidium meyenii*) improve scopolamine-induced memory impairment in mice. Food Chem. Toxicol. 45, 1882–1890.

Rubio, J., Riqueros, M.I., Gasco, M., Yucra, S., Miranda, S., Gonzales, G.F., 2006. *Lepidium meyenii* (Maca) reversed the lead acetate induced—damage on reproductive function in male rats. Food Chem. Toxicol. 44, 1114–1122.

Ruiz-Luna, A.C., Salazar, S., Aspajo, N.J., Rubio, J., Gasco, M., Gonzales, G.F., 2005. *Lepidium meyenii* (maca) increases litter size in normal adult female mice. Reprod. Biol. Endocrinol. 3, 16.

SCIICEX, 2013. Evolución de las exportaciones del producto maca. Sistema Integrado de Información de Comercio Exterior, Peru. http://www.siicex.gob.pe/siicex/apb/ReporteResumen.aspx?psector=1025&preporte=prodresu&pvalor=1934.

Sendker, J., Nahrstedt, A., 2008. Generation of primary amide glucosides from cyanogenic glucosides. Phytochemistry 70, 388–393.

Shrestha, R., Noordermeer, M.A., van der Stelt, M., Veldink, G.A., Chapman, K.D., 2002. N-acylethanolamines are metabolized by lipoxygenase and amidohydrolase in competing pathways during cottonseed imbibition. Plant Physiol. 130, 391–401.

Tang, C.S., Bhothipaksa, K., Frank, H.A., 1972. Bacterial degradation of benzyl isothiocyanate. Appl. Microbiol. 23, 1145–1148.

Ueda, N., Kurahashi, Y., Yamamoto, S., Tokunaga, T., 1995. Partial purification and characterization of porcine brain enzyme hydrolyzing and synthesizing anandamide. J. Biol. Chem. 270, 23823–23827.

Wang, Y., Wang, Y., McNeil, B., Harvey, L.M., 2007. Maca: an Andean crop with multi-pharmacological functions. Food Res. Intern. 40, 783–792.

Williams, D.J., Critchley, C., Pun, S., Chaliha, M., O'Hare, T.J., 2009. Differing mechanisms of simple nitrile formation on glucosinolate degradation in *Lepidium sativum* and *Nasturtium officinale* seeds. Phytochemistry 70, 1401–1409.

Xing-Hao, T., Hua, Z., Hong, Z., Ying, F., Jin, G., Wen-Wen, Z., 2014. Experimental study on fluidized bed drying process of Maca (*Lepidium meyenii* Walp.) tuber. J. Chem. Pharm. Res. 6, 1185–1193.

Yábar, E., Pedreschi, R., Chirinos, R., Campos, D., 2011. Glucosinolate content and myrosinase activity evolution in three maca (*Lepidium meyenii* Walp.) ecotypes during preharvest, harvest and postharvest drying. Food Chem. 127, 1576–1583.

Zhao, J., Muhammad, I., Dunbar, D.C., Mustafa, J., Khan, I.A., 2005. New alkamides from maca (*Lepidium meyenii*). J. Agric. Food Chem. 53, 690–693.