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Investigation of the Tuber Constituents of Maca (*Lepidium meyenii* Walp.)

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Lepidium meyenii, known in South America as maca, has received attention worldwide as a powerful energizer that improves physical and mental conditions and increases fertility. Because of these reports, we investigated the secondary metabolites of the tuber of maca. The methanol extract of the tuber of maca contained, in addition to free sugars and amino acids, the following: uridine, malic acid and its benzoyl derivative, and the glucosinolates, glucotropaeolin and *m*-methoxyglucotropaeolin. Because glucosinolates and their derived products have received increasing attention due to their biological activities, the occurrence of glucosinolate degradation products in the hexane extract was also investigated, and benzylisothiocyanate and its *m*-methoxy derivative were isolated. The two glucosinolates were semiquantified by HPLC, and benzylisothiocyanate was semiquantified by GC/MS. The methanol extract of maca tuber also contained (1*R*,3*S*)-1-methyltetrahydro-β-carboline-3-carboxylic acid, a molecule which is reported to exert many activities on the central nervous system.

KEYWORDS: ***Lepidium meyenii*; maca; Brassicaceae; glucosinolates; methyltetrahydro-β-carboline-3-carboxylic acid**

INTRODUCTION

Lepidium meyenii Walpers (Cruciferae), also reported as *Lepidium peruvianum* Chacon (*1*), known in South America as "maca", is a biennial herbaceous plant that grows almost exclusively in a restricted area of Central Peru, in the Meseta de Bombon near lake Chinchaycocha, at the border of the Junin and Pasco Departments. Maca grows between 3700 and 4500 m above sea level, where low temperature and strong winds limit other crops, but it can be successfully cultivated outside its current habitat. The tuber of this plant was very well appreciated in pre-Inca and Inca times. During the Inca Empire maca consumption was limited to the nobility, the clergy, and the privileged classes; it was also given as a prize to warriors. Nowadays Andean people use the tuber of maca as boiled or roasted food, in soups, or to prepare drinks that are thought to act as a tonic to combat anemia and insomnia and as a regulator of female menstruation and menopause (*1*).

In a previous study, the carbohydrate, lipid, sterol, protein, fiber, amino acid, fatty acid, and mineral composition of the tuber of maca has been determined, and the results showed the high nutritional quality of this tuber (*2*). In particular, the high contents of carbohydrates, proteins, vitamins, and minerals make maca a powerful energizer, particularly indicated in people who are undernourished and stressed, and also to improve physical and mental energy. Furthermore, Andean people are firmly convinced that eating maca increases fertility, and recently athletes have used maca as an excellent alternative to anabolic steroids (*1*). Because of these reports and in continuation of our studies on South American food plants, we investigated the secondary metabolites of the tuber of maca.

MATERIALS AND METHODS

Materials and Reagents. Sinigrin monohydrate and benzylisothiocyanate were purchased from Aldrich Chemical Co. (Milwaukee, WI). Pure HPLC-grade water was generated in the laboratory using a Millipore Milli-Q gradient purification system (Millipore Corp., Bedford, MA). HPLC-grade methanol and hexane were purchased from J. T. Baker (Baker Mallinckrodt, Phillipsburg, NJ).

Plant Material. The pulverized maca root, commercially known as Macandina, was kindly supplied by Naturalfa, Química Suiza, Lima (Peru). Maca was collected from surroundings of the Lake Chinchaycocha (Junin, Perú) and identified as *L. meyenii* Walp. by the Biology Department Herbarium in the Universidad Nacional Agraria (Lima, Peru).

Extraction and Separation of Compounds for Spectral Analysis. The maca root was chopped and dried in a hot air oven (<60 °C, humidity <6%), then was milled, and the resulting powder was homogenized according to the patent-pending process (*3*).

The powdered tuber (500 g) was extracted successively with hexane, chloroform, and methanol at room temperature. The extractive solutions were concentrated to dryness under reduced pressure at 40 °C, giving, respectively, 6.5 g (hexane), 3.0 g (chloroform), and 103.0 g (methanol)

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of dried residues. The hexane extract (3.5 g) was loaded onto a silica gel column (3.5 cm × 120 cm) eluted with hexane (until fraction 92) and increasing amounts of chloroform (2% until fraction 128, 5% until fraction 152, 10% until fraction 190, and 100% until fraction 542). Fractions of 10 mL were collected: fractions 178−188 contained the pure **4** (180 mg), and fractions 319−325 contained the pure **5** (65 mg). The MeOH extract (8.7 g) was partitioned between *n*-BuOH (500 mL) and water (500 mL) giving 0.45 g (*n*-BuOH) and 7.8 g (H_2O) of dried residues. The *n*-BuOH soluble portion was submitted to (68 cm × 1.5 cm) Sephadex LH-20 chromatography using MeOH as eluent, giving 70 fractions of 8 mL. Fractions 34−36 were submitted to RPHPLC on a (300 mm × 7.8 mm i.d., 10 μm) μ-Bondapack C-18 column using MeOH/H_2O (1:3) (2 mL/min) as eluent, and afforded **1** (3 mg, R_t = 8 min); and fractions 45−47 contained malic acid benzoate (5.5 mg). The water soluble portion (2.2 g) was submitted to (68 cm × 2.5 cm) Sephadex LH-20 chromatography using MeOH (2.5 L) as eluent, giving 55 fractions of 8 mL. Fractions 24−32 (406 mg) contained proline, fractions 46−48 (37 mg) contained uridine, fractions 53−55 (24 mg) contained malic acid, and fractions 63−66 (7.7 mg) contained the mixture of glucosinolates. Separation of glucosinolates was performed as reported below.

Spectroscopic Analysis. ^{1}H and ^{13}C spectra were measured on a Bruker DRX-600 spectrometer in CD_3OD, operating at 599.19 MHz for ^{1}H and 150.86 MHz for ^{13}C, using UXNMR software packaging.

Analysis of Nonvolatile Glucosinolates. For nonvolatile glucosinolate analysis, 150-mg portions of powdered and dried plant were extracted twice with 4.5 mL of 70% ethanol for 30 min, with continuous mixing, followed by centrifugation at 3000 rpm for 10 min. The supernatants were combined, and 70% ethanol was added to reach the final volume of 10 mL. This solution was filtered using a 0.45-μm filter.

HPLC separation of nonvolatile glucosinolates was performed using an HP 1100 apparatus equipped with a photodiode-array detector (Agilent, Palo Alto, CA). Samples (200 μL) were injected onto a 300 mm × 7.8 mm i.d., 10-μm μ-Bondapak C-18 semipreparative column (Waters Corporation, Milford, MA) at a flow rate of 4 mL/min. The mobile-phase solvents were HPLC-grade water (A), modified with an ion-pairing reagent consisting of 0.1% triethylamine, pH 7.0, and methanol (B). After a 10 min hold at 0% solvent B, elution was performed by a linear gradient from 0 to 100% solvent B in 60 min to afford compound **2** (R_t = 23.8 min) and compound **3** (R_t = 26.8 min). The column effluent was monitored at 235 nm.

The nonvolatile glucosinolates of *Lepidium meyenii* were semiquantified by HPLC using sinigrin as internal standard, and peak area ratios between the area of each glucosinolate present in the extract and those of sinigrin were calculated and multiplied by the sinigrin concentration. Samples were run in triplicate, with the mean and standard deviation reported.

Nonpolar Compound Analysis. Nonpolar compound analysis was carried out by steeping 1 g of powdered and dried plant with 20 mL of hexane. The hexane extract was filtered and evaporated under a stream of nitrogen. The dry extract was derivatized with diazomethane at room temperature for 10 min, dried, and reconstituted in 2 mL of hexane. A 1-μL sample of this solution was injected into the GC/MS spectrometer.

To semiquantify the benzylisothiocyanate (**4**), 1 μL of the hexane extract was directly injected into the GC/MS without concentration or derivatization of the sample before the analysis because of its high volatility.

Electrospray Mass Spectrometry (ESI/MS). ESI/MS analysis was performed in both positive and negative ion modes using a Finnigan LCQ Deca ion trap instrument (Thermo Finnigan, San Jose, CA) equipped with Xcalibur software. HPLC-purified glucosinolates samples were infused in the ESI source by using a syringe pump; the flow rate was 3 μL/min. The capillary voltage was 5 V, the spray voltage was 5 kV, and the tube lens offset was 35 V. The capillary temperature was 220 °C. Data were acquired in MS and MS/MS scanning mode.

GC/MS analysis. The derivatizated hexane extract was analyzed by GC/MS. The GC/MS system consisted of a GC8000 gas chromatograph (Micromass, Manchester, UK) with a 30 m × 0.25 mm i.d. 0.25-μm, DB-5 capillary column (J and W Scientific, Folsom, CA), which was connected directly to the mass spectrometer via a heated transfer line. The transfer line temperature was maintained at 260 °C. The carrier gas was helium with a flow speed of 1 mL/min, and the injection temperature was 250 °C. The injector was operated in the splitless mode. The oven temperature was programmed at 40 °C for 5 min, then increased to 60 °C at a rate of 10 °C/min. Between 60 and 150 °C the temperature was increased at a rate of 15 °C/min, and finally to 280 °C at increments of 3 °C/min. The mass spectrometer was a TRIO 2000 quadrupole instrument (Micromass, Manchester, UK) set in electron ionization mode. The ion source temperature was 200 °C and the ionization energy was 70 eV. The spectra were acquired from *m*/*z* 30−550, with a scan time of 0.6 s and a interscan time of 0.08 s, using Mass Lynx software to control the GC/MS system, acquisition, and analysis of the data.

The benzylisothiocyanate (R_t = 8.3 min) was semiquantified by GC/MS using external standard calibration. Samples were run in triplicate, with the mean and standard deviation reported.

Glucotropaeolin (**2**). ^{1}H NMR (CD_3OD): δ 7.45 (2H, dd, J = 1.5 and 7.8 Hz, H-2, H-6), 7.36 (2H, dt, J = 1.5 and 7.8 Hz, H-3, H-5), 7.29 (1H, dt, J = 1.5 and 7.8 Hz, H-4), 4.52 (1H, d, J = 6.0 Hz), 4.27 (1H, d, J = 10.0 Hz), 4.09 (1H, d, J = 10.0 Hz), the remaining signals were overlapped in the region between δ 3.12 and 3.85. ESIMS, *m*/*z* 408 $[M - H]^-$. Data were consistent with that reported by Prestera et al. (*4*).

m-Methoxyglucotropaeolin (**3**). ^{1}H NMR (CD_3OD): δ 7.28 (1H, dd, J = 7.8 and 7.8 Hz, H-5), 7.04 (2H, m, H-2, H-6), 6.85 (1H, ddd, J = 1.5, 1.5 and 7.8 Hz, H-4), 4.50 (1H, d, J = 6.0 Hz), 4.26 (1H, d, J = 10.0 Hz), 4.09 (1H, d, J = 10.0 Hz), 3.82 (3H, s, OCH_3), the remaining signals were overlapped in the region between δ 3.12 and 3.85. ESIMS, *m*/*z* 438 $[M - H]^-$ (*4, 5*).

Benzylisothiocyanate (**4**). ^{1}H NMR (CD_3OD): δ 7.41 (2H, dt, J = 1.5 and 7.8 Hz, H-3, H-5), 7.36 (1H, dt, J = 1.5 and 7.8 Hz, H-4), 7.33 (2H, dd, J = 1.5 and 7.8 Hz, H-2, H-6), 4.75 (2H, s, CH_2). EIMS, *m*/*z* 149 $[M]^{+\bullet}$ (*4, 5*).

m-Methoxybenzylisothiocyanate (**5**). ^{1}H NMR (CD3OD): δ 7.28 (1H, dd, J = 7.8 and 7.8 Hz, H-5), 6.87 (2H, m, H-2, H-6), 6.84 (1H, ddd, J = 1.5, 1.5 and 7.8 Hz, H-4), 4.67 (2H, s, CH2), 3.81 (3H, s, OCH3). EIMS, *m*/*z* 179 $[M]^{+\bullet}$ (*4, 5*).

Uridine. EIMS, *m*/*z* 244 $[M]^{+\bullet}$.

Malic Acid. EIMS, *m*/*z* 134 $[M]^{+\bullet}$.

Malic Acid Benzoate. ^{1}H NMR (CD3OD): δ 8.08 (2H, dd, J = 1.5 and 7.8 Hz, H-2′, H-6′), 7.60 (1H, dt, J = 1.5 and 7.8 Hz, H-4′), 7.47 (2H, dt, J = 1.5 and 7.8 Hz, H-3′, H-5′), 5.52 (1H, dd, J = 3.9 and 7.0 Hz, H-2), 3.04 (1H, dd, J = 3.9 and 16.2 Hz, H-3α), 2.89 (1H, dd, J = 7.0 and 16.2 Hz, H-3β). EIMS, *m*/*z* 238 $[M]^{+\bullet}$.

(1R,3S)-1-methyltetrahydro-β-carboline-3-carboxylic acid (**1**). ^{1}H NMR (CD_3OD): δ 7.49 (1H, dd, J = 1.5 and 7.8 Hz, H-5), 7.35 (1H, dd, J = 1.5 and 7.8 Hz, H-8), 7.16 (1H, dt, J = 1.5 and 7.8 Hz, H-7), 7.10 (1H, dt, J = 1.5 and 7.8 Hz, H-6), 4.69 (1H, m, H-1), 3.93 (1H, dd, J = 5.2 and 12.0 Hz, H-3), 3.47 (1H, ddd, J = 1.3, 5.2 and 16.0 Hz, H-4α), 3.03 (1H, ddd, J = 2.5, 12.0 and 16.0 Hz, H-4β), 1.75 (3H, d, J = 6.0 Hz, CH_3). ESIMS, *m*/*z* 231 $[M + H]^+$ (*6, 7*).

RESULTS AND DISCUSSION

The MeOH extract of maca tuber was partitioned between *n*-BuOH and water; the *n*-BuOH soluble portion contained the benzoyl derivative of malic acid and the (1*R*,3*S*)-1-methyltetrahydro-β-carboline-3-carboxylic acid (**1**) (**Figure 1**) (*6, 7*). 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acids arise from a Pictet−Spengler condensation between L-tryptophan and aldehydes. This reaction occurs in foods and is temperature- and pH-dependent (*8*). In the last two decades much attention has been devoted to tetrahydro-β-carbolines because of their biological activities. In particular there has been speculation on their role in the central nervous system where they could function as neuromodulators. Tetrahydro-β-carbolines seem to inhibit the enzyme monoamineoxidase (MAO) and monoamine uptake (*9*), to bind the benzodiazepine receptor (*10*), and to play a role in the etiology of alcoholism (*11*−*13*). In addition, some tetrahydro-β-carbolines are comutagens or precursors of mu-

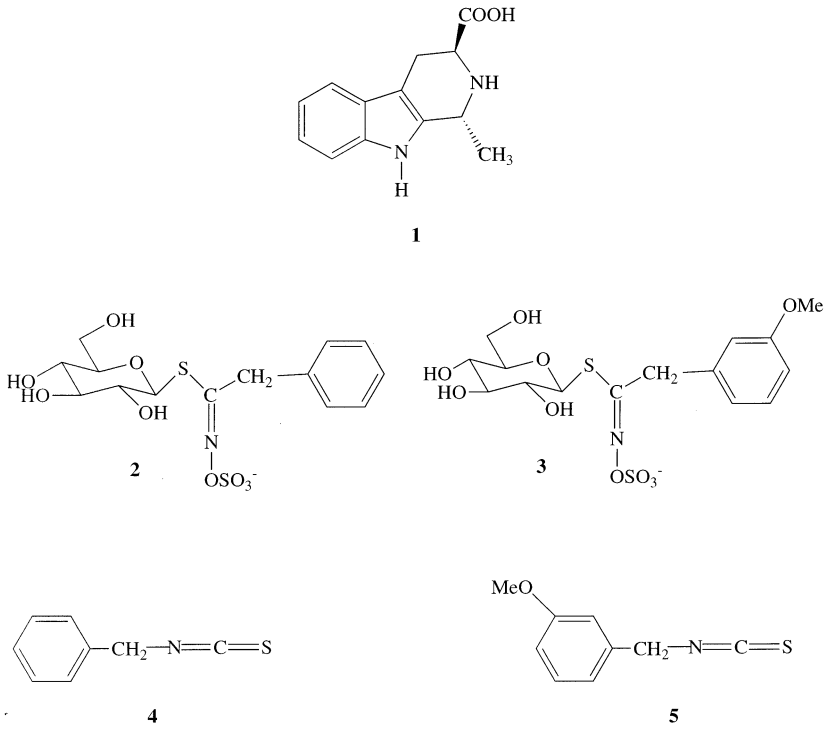

Figure 1. Compounds **1**–**5** from the tuber of maca.

tagens (*14*). They can cause neuronal cell death in vitro (*15*) and can be bioactivated, giving rise to endogenous endotoxins (*16*). On the basis of these data, the occurrence of these compounds in food, in particular in fruits and juice, and their availability during food consumption is an aspect of great interest. Recently, the occurrence of tetrahydro-β-carbolines in chocolate and cocoa has been reported and it has been hypothesized that, on the basis of their activity as mild inhibitors of MAO, they can potentiate the effect of amines (phenylethylamine, tryptamine, serotonin, and others) in chocolate (*17*). Furthermore, chocolate is considered a craved food, and, although the hedonic appeal of chocolate is the predominant factor, it has also been supposed that chocolate contains pharmacologically active substances responsible for the craving. Tetrahydro-β-carbolines, with their neuroactive properties, could play a role in craving (*17*).

The water soluble portion of the maca tuber extract, submitted to Sephadex LH-20 chromatography, yielded (along with free sugars and amino acids with high levels of proline as the main compounds) uridine, malic acid, and a fraction containing glucosinolates. Glucosinolates comprise a group of thioglucosides naturally occurring in various quantities and ratios in the seeds, roots, stems, and leaves of some dicotyledonous angiosperms; cruciferous vegetables are the main source of glucosinolates in human diet (*18*, *19*).

In the intact cells, glucosinolates are kept separate from the endogenous enzyme myrosinase, a β-thioglucoside glucohydrolase able to catalyze the hydrolysis of glucosinolates. When myrosinase and glucosinolates react together after cell disruption, they result in the production of D-glucose and a series of different compounds (i.e., isothiocyanate, thiocyanate, and nitriles) depending on the substrate and the reaction conditions used, such as pH, temperature, and substrate structure (*20*). Glucosinolates and their derived products, generally considered detrimental compounds, have recently received increasing attention because of their biological activities, in particular their ability to play a protective role against cancer (*21*, *22*). The cytotoxic mechanism of these compounds is not completely understood, although it seems that these minor dietary constituents could be inhibitors of carcinogenesis acting by neutralization of a wide number of carcinogens or by the suppression of proliferative activity of neoplastic cells (*23*). Thus, in the first case, glucosinolates and derived products would prevent carcinogen molecules from reaching the target site or interacting with the reactive carcinogenic molecules, or activating important hepatic enzymes for protection against several carcinogens, such as quinone reductase, glutathione S-transferase, and UDP glucuronosyl transferase (*24*). In the second case, they may weaken the effects of genetic changes that occurred in the early stages of neoplastic transformation (*25*). In addition, other studies demonstrated that some compounds, such as isothiocyanates, are able to inhibit protein synthesis and affect carbohydrate metabolism (*26*).

The above results prompted us to identify the glucosinolates occurring in maca tuber and to evaluate their content. The nonvolatile glucosinolates were separated by high-performance liquid chromatography using a mobile phase modified with a buffer to increase retention on the column. The UV chromatogram of the 70% ethanol extract showed the presence of two major peaks that were collected and submitted to NMR and mass spectrometric analysis.

The ^{1}H NMR spectrum of compound **2** was consistent with the glucosinolate glucotropaeolin (*4*, *5*). The ^{1}H NMR spectrum of compound **3** differed from that of **2** in the aromatic proton pattern, and the presence of a signal at δ 3.82, typical of a methoxy group, and was identified as the glucosinolate *m*-methoxyglucotropaeolin, known as glucolimnanthin (*4*, *5*) (**Figure 1**).

To confirm these structures the two major compounds were submitted to mass spectrometric analysis. The negative ion ESI spectrum (NIES) of compound **2** exhibited a strong signal at *m*/*z* 408, due to glucotropaeolin, whereas compound **3** showed

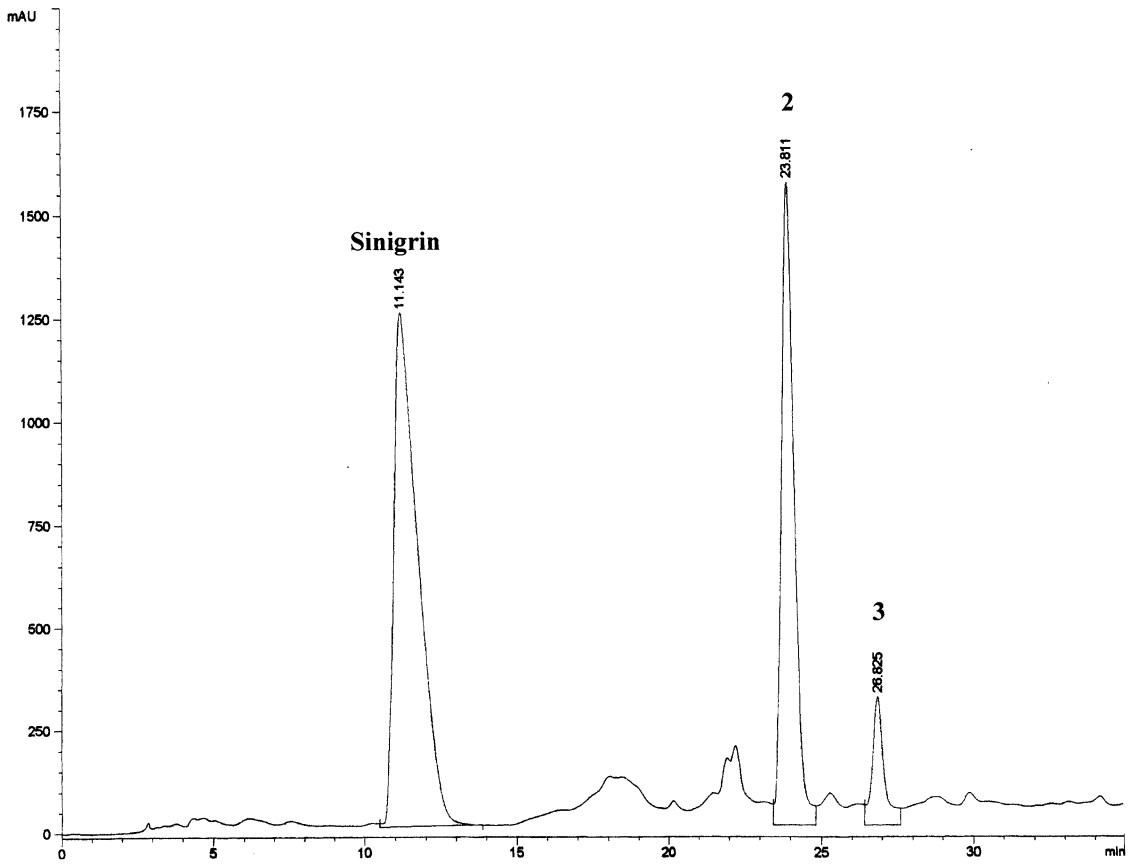

Figure 2. HPLC analysis of glucosinolates from the tuber of maca. Sinigrin (internal standard); **2**, glucotropaeolin; **3**, *m*-methoxyglucotropaeolin.

a signal at *m*/*z* 438, corresponding to a 30-Da increase over the mass value of glucotropaeolin, that confirm the previous NMR results.

The two glucosinolates were semiquantified by HPLC (**Figure 2**). Three aliquots of 70% ethanol extract were analyzed in order to semiquantify the glucosinolates content. It is worth noting that an internal standard was introduced before the extraction to improve the precision of the semiquantitative analysis. The content of **2** and **3** resulted to be 17.6 mg/g of dry tuber (mean $n = 3$; SD = 0.13) and 3.6 mg/g of dry tuber (mean $n = 3$; SD = 0.07), respectively.

The occurrence of the glucosinolates **2** and **3** in the MeOH extract of the tuber of maca prompted us to investigate the presence of the corresponding degradation products in the hexane extract. Thus, this extract was submitted to silica gel column chromatography, affording compounds **4** and **5**. The ^{1}H NMR spectra and EIMS fragmentation patterns were consistent with benzylisothiocyanate (*4*, *5*) and *m*-methoxybenzylisothiocyanate respectively (*4*, *5*).

Benzylisothiocyanate was semiquantified by GC/MS using external standard calibration, and it resulted to be 32.2 ng/g of dry tuber (mean $n = 3$; SD = 0.09).

Glucosinolate content in Cruciferous vegetables is highly variable, depending on plant age and environmental factors that cause the broad range of values reported for vegetables of the same variety (*18*). For example, the average glucosinolate content ranges from ~160 to >250 mg/100 g fresh weight for Brussels sprouts, from ~40 to ~80 mg/100 g fresh weight for cauliflower, from ~40 to ~90 mg/100 g fresh weight for white cabbage, from ~30 to ~95 mg/100 g fresh weight for red cabbage, from ~60 to >200 mg/100 g fresh weight for Savoy cabbage, and from ~10 to ~70 mg/100 g fresh weight for radish. Glucosinolate content in *Brassica* vegetable is reported to be generally about 1% of dry weight (*27*); thus, glucosinolate content in maca is comparable to that of *Brassica* vegetables, and maca could be consumed with other cruciferous vegetables of more common use as a source of glucosinolates in human diet.

Our finding of glucotropaeolin and *m*-methoxyglucotropaeolin as glucosinolates occurring in maca tuber is not consistent with the report of Genyi et al. (*28*) who identified glucosinolates of maca as glucotropaeolin and *p*-methoxyglucotropaeolin. On the other hand, the occurrence of glucotropaeolin and *m*-methoxyglucotropaeolin is in good agreement with the presence of phenylacetonitrile and 3-methoxyphenylacetonitrile, the glucosinolate degradation products, as the main components in the essential oil of the aerial parts of *L. meyenii* (*29*).

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