

Macamides and fatty acids content comparison in maca cultivated plant under field conditions and greenhouse

[Comparación del contenido de macamidas y ácidos grasos en plantas de maca cultivadas en condiciones de campo e invernadero]

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Abstract

Maca (*Lepidium meyenii* Walp., Brassicaceae) is a Peruvian tuberous crop cultivated in the Andes mountains at altitudes above 3.500 m. It is grown for consumption of its nourishing hypocotyl and it is extensively used for nutritional and medicinal purposes. Maca is normally used to increase physical energy, support the immune system, and is traditionally considered to be an aphrodisiac which enhances fertility in humans and in domestic animals. The purpose of the study was to reveal how maca responds to diverse climatic conditions and what the prospects of its cultivation are out of its original area. The macamide (main quality marker of maca) content has been analyzed by HPLC-UV in plant material of various samples of maca of Peruvian origin and it was compared to content in samples of maca cultivated in the Czech Republic, under field conditions and in a greenhouse. There was a significantly lower concentration of macamides in the sample grown in the Czech Republic compared to the Peruvian samples. There were no macamides found in samples cultivated in the greenhouse. If we admit that macamides are responsible for fertility enhancing properties of maca, we can conclude there is no possibility of maca cultivation in the Czech Republic for this use.

Keywords: *Lepidium meyenii* Walp., HPLC, petrolether extract.

Resumen

La maca (*Lepidium meyenii* Walp., Brassicaceae) es una planta de origen peruano y es cultivada en los Andes a alturas sobre los 3.500 m. Se cultiva para el consumo de su hipocotilo que tiene un alto valor nutritivo y propiedades medicinales. La maca aumenta la energía física, refuerza el sistema inmunológico y es considerada como un afrodisíaco que mejora la fertilidad en los humanos y animales. El objetivo del presente estudio fue realizar un seguimiento de la respuesta del cultivo de la maca a diversas condiciones climáticas y de sus perspectivas de cultivo fuera del lugar de origen. El contenido de macamidas (principales marcadores de calidad de la maca) ha sido analizado por HPLC-UV en el material vegetal de diversas muestras de maca de origen peruano y se comparó con el contenido en las muestras de maca cultivada en condiciones de campo e invernadero en la República Checa. Se determinó una concentración inferior, significativamente, de macamidas en las muestras cultivadas en campo en la República Checa en comparación con las muestras peruanas, y no se determinó ningún tipo de macamidas en las muestras cultivadas en invernadero. Considerando que las macamidas tienen un efecto positivo en el mejoramiento de la fertilidad, llegamos a la conclusión de que el cultivo de la maca, para este objetivo, no es apto en condiciones climáticas de la República Checa.

Palabras Claves: *Lepidium meyenii* Walp., CLAR, extracto de etér de petróleo.

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List of abbreviations: HPLC-UV – High-Pressure Liquid Chromatography with UV Detector; RT – retention time

INTRODUCTION

Maca (*Lepidium meyenii* Walp.) is one of the oldest crop of the Andean area. Primitive cultivars of maca were even found in places dating back to 1600 years before Christ and for the indigenous inhabitants it is a vital and valuable commodity up to this day (León, 1964). Maca grows normally at 3500 m above the sea level and often reaching 4450 m (León, 1964; Tello *et al.*, 1992). It is cultivated on very poor and rocky soil and it grows in extreme weather conditions such as freezing, fierce winds and intensive sunlight (Flores *et al.*, 2003). The aerial parts of maca form a rosette of 12-20 leaves and the foliage form a mat. The main stem is reduced while the underground part is a storage organ formed by the taproot and the lower part of the hypocotyl (León, 1964). The fleshy hypocotyls of maca are harvested from June to August and they are rich in sugars, amino acids, vitamins and minerals (Tello *et al.*, 1992). The maca hypocotyls are eaten fresh, cooked or they are stored dried for later consumption. Dried roots are eaten after boiling in water or milk, mixed with fruits or sugarcane rum for cocktails (Johns, 1981; Tello *et al.*, 1992). Maca leaves are usually left on the field after hypocotyl harvest. However, dried leaves are sometimes fed to livestock and guinea-pigs (Clément *et al.*, 2010a). Maca was used for centuries by original Peruvian populations as a traditional folk medicament to enhance vitality and treat sterility of humans and domestic animals (Canales *et al.*, 2000). However, it has many other traditional therapeutic uses, e.g. as a laxative and for the treatment of rheumatism, respiratory problems, premenstrual discomfort, and menopausal symptoms (Kilham, 2000). Knowledge of this highly valued plant and its activities was transferred from generation to generation and during Spanish colonization, the native people even used maca as currency (Chacón de Popovici, 1997). The fertility and libido enhancing effect of maca was confirmed in several studies.

These studies among others proved that maca enhances libido and fertility by modulating the hypothalamus-pituitary axis, regulating hormone secretion, and regulating interaction between the glucosinolates and androgen receptor (AR) (Bogani *et*

al., 2006). It also improves epididymal sperm count and daily sperm production in rats (Gasco *et al.*, 2007; Gonzales *et al.*, 2006). In a study on nine young adults to evaluate the effect of a 4-month oral treatment with maca tablets (1.500 or 3.000 mg/d) on seminal analysis, a significantly increased seminal volume, sperm count per ejaculum, motile sperm count, and sperm motility without modification in sexual hormone serum was observed (Gonzales *et al.*, 2001). Two randomized, doubleblind trials confirmed these findings (Gonzales *et al.*, 2002; Gonzales *et al.*, 2003). After 14 days maca extract supplementation improved 40 km cycling time trial performance and sexual desire in trained male cyclists (Stone *et al.*, 2009). Two randomized controlled trials suggested a significant positive effect of maca on sexual dysfunction and sexual desire in healthy menopausal women and healthy adult men (Zenico *et al.*, 2009; Brooks *et al.*, 2008).

Recent studies confirmed that aqueous or methanol extract of maca roots has the capacity to scavenge free radicals and protect cells against oxidative stress and these aqueous extracts protect the skin of rats against UV irradiations (Lee *et al.*, 2005; Sandoval *et al.*, 2002; Gonzales-Castaneda & Gonzales, 2008). Study of Gonzales *et al.* (2005) confirmed reduced risk of prostate cancer in rats after treatment of aqueous extracts of maca. Several studies demonstrate maca can combat osteoporosis and osteoarthritis (Gonzales *et al.*, 2010; Zhang *et al.*, 2006; Mehta *et al.*, 2007).

In support of related studies the products of maca are increasingly becoming popular in the western world as tonics and food supplements. Dried maca hypocotyls contain several classes of secondary metabolites of interest including alkaloids, amino acids, glucosinolates, fatty acids and macamides, biologically active aromatic isothiocyanates, that have so far been found only in maca (Zheng *et al.*, 2000; Dini *et al.*, 1994; Ganzera *et al.*, 2002; Piacente *et al.*, 2002). Some of the aphrodisiac activities of maca have been related to lipidic fraction of maca, which contains mainly fatty acids and macamides (Zheng *et al.*, 2000), therefore content of macamides is used as the main *quality markers* in maca products. Using HPLC-UV-MS/MS, the main macamides have been identified as *n*-benzylhexadecanamide, *n*-benzyl-(9Z)-octadecanamide, *n*-benzyl-(9Z, 12Z)-octadecadienamide, *n*-benzyl-(9Z, 12Z, 15Z)-

octadecatrienamide and *n*-benzyloctadecanamide (McCullom *et al.*, 2005).

Although maca is adapted to high altitudes and extremely low temperatures, it can be successfully transplanted to the Peruvian seacoast (Chacón de Popovici, 1997) and be cultivated outside its natural localities (Quirós *et al.*, 1996). In lower altitudes, such as in Germany, maca does not form hypocotyls (ACS TRADING CORPORATION, 2012). In the Czech Republic, hypocotyls were formed only in field, not in greenhouse and differences in chemical composition (higher content of proteins and nitrates and lower content of saccharides) compared to commercial maca powder were observed (Lebeda *et al.*, 2003). It is not completely known how climate changes influence its spectrum of components.

The fact that maca plants are already available at Czech markets (STARKL company), caused the demand for further study how the plant might respond to diverse climatic conditions outside the area of its origin. In the present study, planting experiment was carried out at the field conditions and greenhouse in Czech Republic to test the hypothesis that climate changes contain of quality markers, macamides and linoleic and linolenic acids in maca hypocotyls and leaves. Plants were grown under the following conditions:

Climate in Junín district, Peru, origin of maca (Sandoval *et al.*, 2000):

- Altitude: 4100 – 4150 m
- Approximate precipitation: 900 – 1000 mm annual
- Approximate temperature: day average: 11,5° C, night average: 1,8° C
- Approximate sunshine: 110 - 190 hours a month

Climate in Prague, Czech Republic, site of experiment (Prague-guide, 2012):

- Altitude: 257 m
- Approximate precipitation: 590 mm annual
- Approximate temperature: day average: 12,7° C, night average: 4,6° C
- Approximate sunshine: 39 - 230 hours a month

MATERIALS AND METHODS

Plant material

Five samples of dried ground maca hypocotyls and one sample of dried ground leaves were compared.

Three samples of maca hypocotyls came from Peru, these samples were obtained at a local market in

Cuzco (no label, origin in surrounded mountainous areas, sample *a*), in a Peruvian supermarket in Lima (producer: ECOANDINO, origin: Ciudad de Junín, sample *b*) and in a Czech herbal store (producer: AGROINDUSTRIAS FLORIS S.A.C., origin: Junín district, sample *c*) respectively.

Samples of maca cultivated in the Czech Republic were grown in an experimental field (sample *d*) and in greenhouse (sample *e* = hypocotyl, *f* = leaves) of the Czech University of Life Sciences Prague (CULS). The samples grown in the field of CULS were sowed in February 2007 in a greenhouse, transplanted to the field and harvested in October of the same year. In total 78 plants were spaced 30 cm apart and protected from strong sun and slugs. After the harvest, the bulbs were washed, weighed, cut, dried and ground. The samples were dried in a forced air oven (40°C) and then stored in hermetic browned glass bottles at room temperature in the dark. The length of growth of the plants was approximately the same as in Peru. There were 16 plants kept in the greenhouse and left to flower and produce seeds. The seeds were sown in February 2008, 50 grown plants were kept whole growing period in the greenhouse, and hypocotyls and leaves were harvested in October (samples *e*, *f* respectively).

Methods

One gram of each finely ground maca sample was extracted in petrolether in enclosed extraction vials for 4 hours. The solvent was replaced three times and the obtained extract was filtered and evaporated to dryness in a vacuum in a rotary evaporator at 40° C. Each sample was stored dried and before the analysis each one was diluted to final concentration 0.5 mg/mL. Before extraction, internal standard linoleic acid methylester was added in concentration 1 mg/g of dried sample. Analysis was performed by the method described by McCullom *et al.* (2005) and macamides and fatty acids were identified by retention time (RT) by comparison and UV spectra (Figure 1) according to their publication. HPLC conditions are shown in Table 1.

Acquired data were quantified according to the calibration curve obtained for structurally similar methylester of linoleic acid (Sigma Aldrich, CZ).

Analysis were repeated three times and results were expressed as mg/g of dried weight (DW).

Chemicals

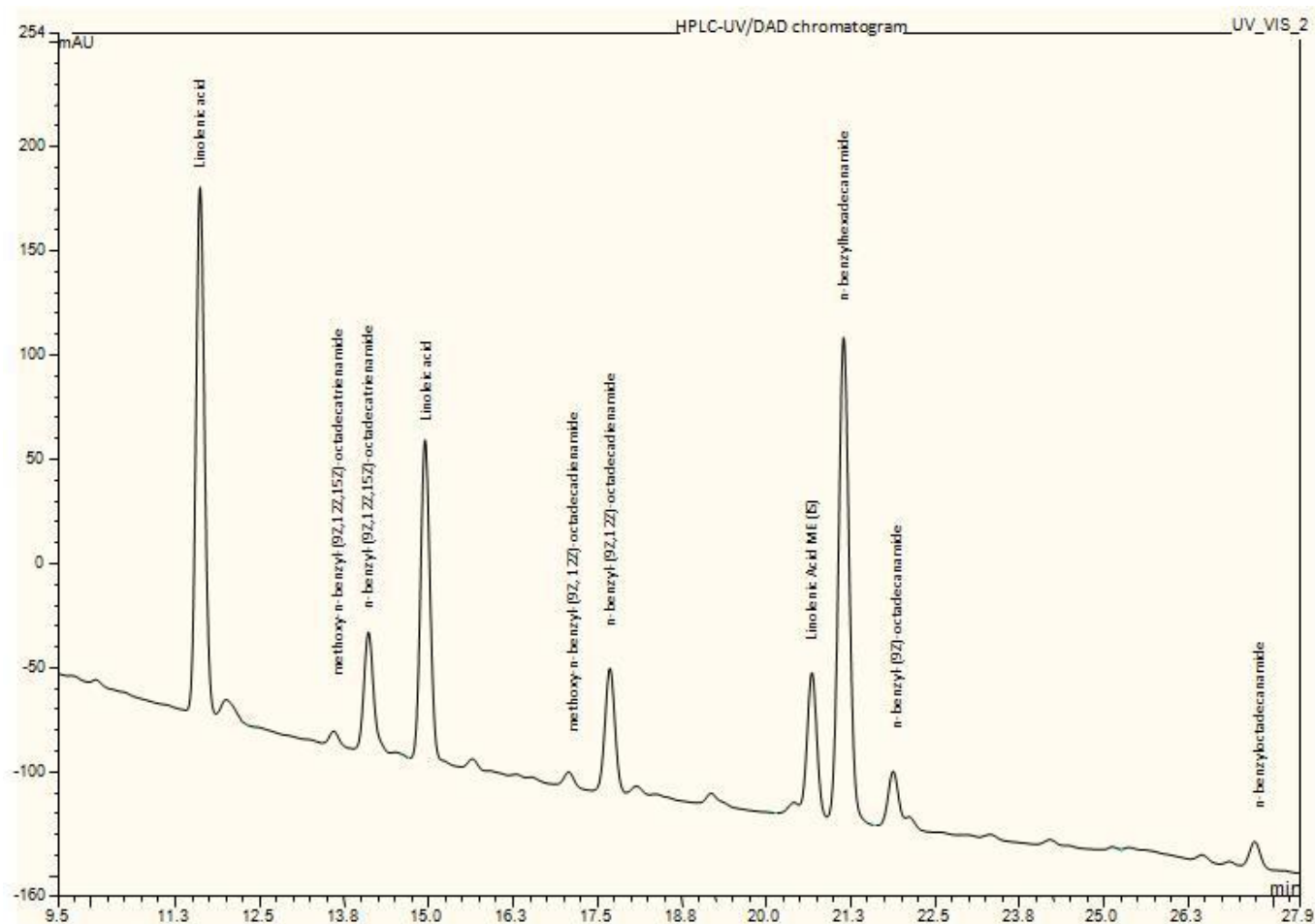
Linoleic acid methylester, trifluoroacetic acid (TFA) purchased from Sigma Aldrich (CZ) while petrol ether and Acetonitril were purchased from Lach-Ner (CZ).

Statistical analysis

Each sample was carried out in triplicates. Results were expressed as means \pm standard deviation. Data were compared using Tukey's test ($p < 0.05$).

Figure 1

HPLC-UV/DAD chromatogram of a petroleum ether extract of ground maca hypocotyls and leaves showing the separation of macamides and fatty acids.



RESULTS AND DISCUSSION

Maca has been widely studied (Bassols *et al.*, 2010; Castaño-Corredor, 2008; Valentova *et al.*, 2008). Seven main macamides were found in most of the samples: n-benzylhexadecanamide (nBED), n-benzyl-(9Z)-octadecanamide (nB9OD), methoxy-n-benzyl-(9Z, 12Z)-octadecadienamide (MnBOD), n-

benzyloctadecanamide (nBOD), n-benzyl-(9Z,12Z,15Z)-octadecatrienamide (nBODT), n-benzyl-(9Z,12Z)-octadecadienamide (nBODD), methoxy-n-benzyl-(9Z,12Z,15Z)-octadecatrienamide (MnBODT), total macamides (TM) were than calculated as sum of each of them (Table 2). Linoleic acid (LA), Linolenic acid (LnA), were also measured

and Linolenic Acid methylester (LnAME) was used as internal standard (IS) (Table 3).

Table 1
HPLC system and condition

<i>Instrument</i>	HPLC Dionex Summit, UVD 340 interfaced with Waters 717 autosampler
<i>Column</i>	Gemini C18 column, 5 μ m, 110A, 4.6 x 250 mm (Phenomenex)
<i>Mobile phase</i>	A: 0.1% TFA in H ₂ O B: Acetonitril, gradient from 20% A to 80% B in 24 min
<i>Flow rate</i>	0.8 mL/min
<i>Detection wavelength</i>	210 nm
<i>Injection volume</i>	20 μ L
<i>Column temp</i>	40° C
<i>Run time</i>	24 minutes gradient with subsequent return to original conditions in 2 min and 10 min postcolumn equilibration, total 36 min.

Content of macamides in samples of maca is shown in Table 2. Expectedly, content of macamides varied among samples. A difference was observed in the total amount of macamides between the samples of Peruvian and Czech origin. From the three Peruvian samples the produced by ECOANDINO contained double the total amount of macamides than the other two, which contained very similar amount. Sample *d*,

Czech ones, cultivated under field conditions had approximately 6 times lower content of macamides than the Peruvian ones and there were no macamides found in hypocotyls of maca grown in the greenhouse. In leaves obtained from plants grown in the greenhouse the content of macamides was 10/20 times lower than in the Peruvian samples.

Table 2
Content of main macamides in maca samples (a – f) expressed in mg/g DW, RT (retention time in min), Values are the mean of 3 determinations, P < 0.05.

compound	TM	MnBODT	nBODD	nBODT	nBOD	MnBOD	nB9OD	nBED
RT		13.60	17.69	14.11	27.24	17.08	21.89	21.16
a	3.77	0.05±0.01	0.71±0.08	0.46±0.05	0.13±0.02	0.08±0.01	0.31±0.02	2.03±0.15
b	6.21	0.09±0.02	0.88±0.13	0.82±0.11	0.20±0.04	0.11±0.03	0.43±0.04	3.68±0.21
c	3.14	0.04±0.01	0.70±0.06	0.56±0.04	0.14±0.01	0.06±0.01	0.25±0.03	1.39±0.12
d	1.05	0.02±0.01	0.55±0.04	0.21±0.02	0.07±0.02	0.06±0.01	0.05±0.01	0.09±0.02
e	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
f	0.38	n.d.	0.70±0.01	0.14±0.02	n.d.	n.d.	n.d.	0.70±0.01

More in detail sample *b* possesses the highest amount of all identified macamides, nBODD is the one that shows the lowest difference in concentration among Peruvian samples.

Content of main fatty acids in samples of maca is shown in Table 3. As demonstrated by the quantification of macamide content, also fatty acid content varied among samples. A difference was

observed in both LA and LnA between the samples of Peruvian and Czech origin. Among the three Peruvian samples the *c*, produced by AGROINDUSTRIAS FLORIS contained nearly nine times LnA than sample grown in the greenhouse. Even if LA content was only two times higher. LA content demonstrated to be much closer among samples, in fact it ranged between 1.87 mg/g DW of *e* sample and 3.43 mg/g DW of *c*

sample. Leaves demonstrated to possess the lowest LA content, 1.18 mg/g DW (Table 3).

The study proved that maca produced much less macamides (quality markers of maca products) under mild climatic conditions of the Czech Republic than in its original environment, wind-blown rocky areas in high plateau of Peru (Kilham, 2000). Moreover the size of the storage organ of maca cultivated within this study in the Czech Republic was much smaller than if cultivated in the original

environment, while hypocotyls of plants cultivated in the greenhouse were even smaller than that from plants in the field, the same conclusion was reported by Lebeda in 2003. The hypothesis, that the day length would affect the formation of the storage organ of maca, was disproved in field trials in California in 1997, therefore we can exclude significant role of the day length as a contributing factor (Quirós *et al.* 1997).

Table 3
Content of main fatty acids in maca samples (a – f) expressed in mg/g DW, RT (retention time in min), Values are the mean of 3 determinations, P < 0.05.

compound	LnAME	LnA	LA
RT	20.69	11.62	14.95
a	1.00±0.03	2.46±0.14	2.44±0.19
b	1.00±0.02	3.21±0.26	2.19±0.21
c	1.00±0.05	4.30±0.38	3.43±0.33
d	1.00±0.05	1.60±0.11	2.56±0.21
e	1.00±0.04	0.49±0.06	1.87±0.11
f	1.00±0.04	2.69±0.18	1.18±0.09

Effect of the soil on content of macamides in maca hypocotyls studied by Clément *et al.* (2010a) remains unclear. On the other hand, in later study, where maca was cultivated on two different sites in Peru 217 km apart, Clément *et al.* (2010a and 2010b) mentions importance of the site, which not only significantly affects yield growth, but also the concentration of secondary metabolites, while correlation of yield and content of macamides is positive (Clément *et al.*, 2010b). When we compared yield from the field and the greenhouse and the content of macamides in of each sample we found the same positive correlation. Effect of cultivation history of the site is controversial or absent (Clément *et al.*, 2010b).

CONCLUSIONS

The fact, that maca cultivated in a field in the Czech Republic had few macamides and greenhouse maca had macamides under detection limit, confirmed presumption that cold and unfavorable climatic

conditions increase formation of maca's secondary metabolites.

If we admit that macamides are responsible for fertility enhancing properties of maca, we can conclude there is no possibility of maca cultivation in the Czech Republic, and probably in other countries with the same climatic conditions, for this use. The hint for future investigation is to grow maca in temperate zone under stress conditions in order to induce the secondary metabolites formation.

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