

Avaliação do perfil de minerais, ácidos graxos e bioacessibilidade da diosgenina em diferentes tipos de maca peruana (*Lepidium meyenii* Walp)

Evaluation of fatty acid and mineral profiles and bioaccessibility of diosgenin in different types of Peruvian maca (*Lepidium meyenii* Walp)

Evaluación del perfil de minerales, ácidos grasos y bioaccesibilidad de diosgenina en diferentes tipos de Maca peruana (*Lepidium meyenii* Walp)

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Resumo

O presente trabalho teve por objetivo estudar a bioacessibilidade da diosgenina, ácidos graxos e perfil mineral em diferentes tipos de maca peruana (*Lepidium meyenii* Walp). Foram realizadas análises de minerais, ácidos graxos, saponinas e bioacessibilidade da diosgenina. Para os minerais, os valores foram detectados: cama comercial (MC) 490,65mg / 100g de Ca, 1472,65mg / 100g de K, em cama vermelha (VM) 1478,25mg / 100g de K e 670,25mg / 100g

de Ca, amarelo amarelo (MA) 875,65mg / 100g Ca, 1215,25mg / 100g K. As maiores concentrações de ácidos graxos estavam relacionadas à porção não saturada. % de ácido oleico na maca vermelha. Em relação ao teor de saponina e diosgenina, a serapilheira comercial apresentou os maiores teores de 250,33mg / 100g para saponinas e 340,56µg / ml para diosgenina. Esses resultados enfatizam a importância do consumo desse vegetal rico em nutrientes com ação efetiva no metabolismo humano ($p < 0,05$).

Palavras-chave: Compostos bioativos; Saponinas; HPLC; Bioprospecção; Química de alimentos.

Abstract

The present work aimed to study the bioaccessibility of diosgenin, fatty acid and mineral profile in different types of Peruvian maca (*Lepidium meyenii* Walp). Analyzes of minerals, fatty acids, saponins and bioaccessibility of diosgenin were performed. For minerals the values were detected: commercial litter (MC) 490.65mg / 100g of Ca, 1472.65mg / 100g of K, in red litter (MV) 1478.25mg / 100g of K and 670.25mg / 100g of Ca, yellow yellow (MA) 875.65mg / 100g Ca, 1215.25mg / 100g K. The highest concentrations of fatty acids were related to the unsaturated portion. % oleic acid in the red stretcher. Regarding the saponin and diosgenin content, the commercial litter presented the highest contents 250.33mg / 100g for saponins, and 340.56µg / ml for diosgenin. These results emphasize the importance of the consumption of this nutrient-rich vegetable with effective action on human metabolism ($p < 0.05$).

Keywords: Bioactive compounds; Saponins; HPLC; Bioprospecting; Food chemistry.

Resumen

El presente trabajo tuvo como objetivo estudiar la bioaccesibilidad de diosgenina, ácido graso y perfil mineral en diferentes tipos de maca peruana (*Lepidium meyenii* Walp). Se realizaron análisis de minerales, ácidos grasos, saponinas y bioaccesibilidad de diosgenina. Para los minerales se detectaron los valores: basura comercial (MC) 490.65mg / 100g de Ca, 1472.65mg / 100g de K, en basura roja (MV) 1478.25mg / 100g de K y 670.25mg / 100g de Ca, amarillo amarillo (MA) 875.65mg / 100g Ca, 1215.25mg / 100g K. Las concentraciones mayores de ácidos grasos estaban relacionadas con la porción insaturada. % de ácido oleico en la camilla roja. Con respecto al contenido de saponina y diosgenina, la camada comercial presentó los mejores contenidos de 250,33 mg / 100 g para saponinas y 340,56 µg / ml para

diosgenina. Estos resultados enfatizan la importancia del consumo de este vegetal rico en nutrientes con acción efectiva sobre el metabolismo humano ($p < 0.05$).

Palabras clave: Compuestos bioactivos; Saponinas HPLC; Bioprospección; Química de los alimentos.

1. Introduction

Bioavailability can be defined as the proportion of an ingested nutrient available for use or store by the body (Castenmiller & West, 1998). For humans, this is defined as the fraction of ingested nutrients transferred during digestion to micelles and absorbed by intestines (Stahl et al., 2002).

The study of the accessibility of nutrients in food is thus greatly important to human health since these nutrients are required for human metabolism. Consumption of Peruvian maca (*Lepidium meyenii* Walp) is associated with hormonal secretion regulation, metabolism stimulation, memory improvement, and action against depression, anemia, leukemia, AIDS, cancer, and alcoholism (Quiros & Aliaga, 1997; Cárdenas, 2005).

The chemical composition of this tuber includes a variety of secondary metabolites from different pathways, including glucosinolates, phenylpropanoids (polyphenols), isoprenoids (monoterpenes and sesquiterpenes), and alkaloids (Dini et al., 2002; Piacente et al., 2002; Sandoval et al., 2002; Tellez et al., 2002). In addition, Peruvian maca contains a variety of phytochemicals like campesterol, stigmasterol, betasitosterol, benzyl isothiocyanates, catechins, and various glucosinolates (Zheng et al. 2000; Li et al. 2001) that have shown significant potential as antioxidants (Sandoval et al., 2002).

Different varieties of maca are described by hypocotyle color, which can appear red, yellow, or black (Tello & Calderon 1992; Yllescas 1994). In addition, maca varieties show significant differences in levels of minerals, proteins, and nutrients such as potassium and iron (Gonzales et al., 2006; Rubio et al. 2006).

Maca has also been used as a portion of human health-promoting food, resistance builder, and fertility promoter due to its regulation of hormone metabolism and secretion, memory enhancement, and antidepressant activities (Zhang et al. 2017). In addition, significant improvements in hormonal modulation and oxidative stress have been observed after maca ingestion, which may be related to the levels of saponins, alkaloids, steroid hormones, and polyphenol compounds within maca (Omrah et al., 2010; Tang et al., 2017).

The beneficial effects of maca consumption may thus be correlated with the levels of saponins, especially diosgenin. Although saponins are considered antinutritional, they also possess show several health-friendly biological properties, including antibacterial, antifungal, antioxidant, cholesterol-lowering, and anticancer activities (Shi et. al., 2009; Wang et. al., 2007).

Diosgenin is a sapogenin that is structurally similar to cholesterol and other steroids and is the main precursor in the production of synthetic steroids in the pharmaceutical industry, serving as an important starting material for the production of corticosteroids, sex hormones, oral contraceptives, and anti-inflammatory drugs, (Balamurugan et al, 2013). Given the above information, the objective of this work was sought to evaluate the diosgenin bioaccessibility and fatty acid profiles in different types of litter (*Lepidium meyenii* Walp).

2. Material and Methods

The research is explanatory and experimental, with part conducted in the field where the fruits were collected and in the Chemical Food Analysis Laboratory, of the National Institute of Research in the Amazon (Pereira et al., 2018).

Obtaining the raw material

Three 10 kg lots of maca (*Lepidium meyenii* Walp) were obtained from Cuzco, Peru, stored in a sealed Styrofoam box, and transported to the Food Physicochemical Laboratory (LFQA) of the Environment and Health Society Coordination (COSAS) at the National Research Institute of Amazonia (INPA) for analysis.

2.1 Maca sample analysis

2.1.1 Fatty acids

Pulp was extracted using an automatic pulp remover (Itametal, 1.5 mm mesh) and homogenized in a blender prior to fatty acid quantification and analysis. Total lipids were extracted and identified according to protocols from Bligh & Dyer (1959). Fatty acids in oil were converted to fatty acid methyl esters then analyzed using a Shimadzu model gas chromatography (GC) for Mass Spectrometer Gas Chromatograph/GC- 2010 PLUS (Kyoto,

Japan) equipped with a flame ionization detector. Compounds were separated on a 30 m RTxR-5 capillary fused silica column with an internal diameter of 0.25 mm and a film thickness of 0.25 μ m. Operating conditions were as follows: programmed column temperature, 80–220°C (5°C/min); injector temperature, 230°C; detector temperature, 240°C; carrier gas, hydrogen; gas linear velocity, 40 cm/s; sample ratio, 1:50. Fatty acids were identified by comparing retention times with those of standards and were quantified by normalizing peak areas. Fatty acid standards used (Sigma Supelco) were hexanoic acid (C6:0), octanoic acid (C8:0), decanoic acid (C10:0), undecanoic acid (C11:0), duodecanoic acid (C12:0), tetradecanoic acid (C14:0), cis-9-tetradecenoic acid (C14:1), hexadecanoic acid (C16:0), cis-9-hexadecenoic acid (C16:1), acid heptadecanoic acid (C17:0), 8-heptadecanoic acid (C17:1), octadecanoic acid (C18:0), trans-9-octadecaenoic acid (C18:1), cis-9-octadecenoic acid (C18:1), cis-9 acid, trans-11-octadecaenoic acid (C18:2), cis-9, cis-12-octadecadienoic acid (C18: 2), 9,12,15-octadecatrienoic acid (C18: 3), 6,9,12-octadecatrienoic acid (C18:3), acid eicosanoic acid (C20:0), cis-9-eicosenoic acid (C20:1), acid 8,11-eicosadenoic acid (C20:2), 5,8,11-eicosatrienoic acid (C20:3), docosanoic acid (C22:0), 5,8,11,14,17-eicosapentaenoic acid (C20:5), cis-13-docosenic acid (C22:1), tetracosanoic acid (C24:0), cis-15- tetracosenoic (C24:1).

2.1.2. Chemical composition

Moisture, ash, and protein content was determined the protocol by the Association of Official Analytical Chemists (AOAC) (2016) using a 6.25 factor to convert nitrogen percentages into protein content. Total lipids were extracted and identified according to Bligh and Dyer (1959) as described above. Total carbohydrates were calculated using the following equation: $c = 100 - (\text{Moisture} + \text{Lipid} + \text{Protein} + \text{Ash})$. Caloric value was determined by using an indirect method based on main product nutrient (carbohydrates, protein, and lipids) conversion factors.

The mineral content was determined in triplicates using atomic absorption spectrometry method recommended by Adolfo Lutz Institute (IAL, 2008), and according to Varian manual (2000). The digestion of samples was performed using microwave in MARS - Xpress CEM Corporation, MD - 2591 digester followed by mineralization of organic matter using concentrated nitric acid, cooling and dilution with deionized water, and reading. Reading was performed directly using diluted atomic absorption spectrophotometer solutions (Spectra AA, model 220 FS, Varian, 2000), with specific lamps according to the manufacturer's manual.

The quantified mineral elements were Ca, K, Na, Mg, Fe, Zn, Mn, and Cu. For controlling the analyses, recommendations according to Cornelis (1992) were used, having certified reference material *Peach leaves* (NIST-SRM 1547).

2.1.3. Extraction and quantification of total saponins

Sprayed drug was weighed and 0.2 g degreased with 30 mL hexane for 2 h then powder filtered and oven dried. Powder was refluxed with three 20 mL aliquots of methanol-water (4:1) for 30 min and the methanolic extract filtered and concentrated via extraction with three 20 mL volumes of water-saturated n-butanol. The butanolic fraction was collected and concentrated until dried then dissolved in 100 mL of water. Next, 1 mL 0.2% cobalt chloride and 1 mL concentrated sulfuric acid were added to 1 mL aliquot of resuspended butanolic solution. Absorbance of both processed methanolic and butanolic extracts was then measured at 284 nm (Vigo et al. 2003). A calibration curve consisting of six points was then constructed, producing a correlation coefficient $R^2 = 0.9904$, confirming reading linearity and indicating that the technique was performed correctly. The saponin analysis was performed using a spectrophotometer and following recommendations from the literature. The standard curves of Merck saponins were generated at five concentrations: 0.08 mg/mL, 0.12 mg/mL, 0.16 mg/mL, 0.20 mg/mL, 0.28 mg/mL, 0.36 mg/mL, and 0.40 mg/mL. The chromogenic reagent used was 0.2% cobalt chloride with an optimal reaction time of 10 to 20 min. A wavelength of 284 nm was used for analysis.

2.1.4. Diosgenin quantification

Diosgenin quantification was performed according to Desai et al (2015). Analysis was performed using an HPLC apparatus with a mobile phase of acetonitrile and water (92:8, v/v) with a flow rate of 1 mL/min. Detection was done at 203 nm with column equilibration at 25°C and an injection volume of 20 µL. To prepare the standard solution, Sigma-Aldrich diosgenin (100 mg) was diluted to a concentration of 1000 µg/mL in 100 mL of methanol using ultrasound for 10 min. Several aliquots of this standard solution were then used to create standard solutions of varying concentrations. Dried maca extracts were obtained by the method mentioned above and 10 mg of dried extract diluted in 10 mL of methanol using ultrasound for 10 min then filtered through a 0°C PTFE filter with a 2 µm pore size. To assess the specificity and suitability of the method, two blank (methanol) injections, six individual

standard solution injections (100 µg/mL), and two sample solution injections were performed prior to experimental measurements. Any interference found in blank or experimental samples was checked and relevant parameters, including resolution, asymmetry, and repeatability of the signal area, was evaluated.

To determine the linearity ratio, aliquots of diosgenin stock solution were diluted in methanol to concentrations of 1 µg/mL, 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, 50 µg/mL, and 60 µg/mL. Measurements were performed in duplicate and chromatograms adjusted to 203 nm. Quantification was performed by maintaining peak area and compound concentrations based on the calibration curve and thus establishing correlation coefficients.

2.2 Statistical analysis

Statistical analysis of chemical and physical variables was performed using the SISVAR program (Ferreira, 2010). The multivariate statistical analysis via PCA and HCA techniques with Sensomaker software, version 1.91 (Nunes & Pinheiro, 2017) was using for analyzed the mineral and diosgenin composition profiles.

3. Results and Discussion

3.1. Fatty acid content

Table 1 shows fatty acid concentrations in maca varieties (commercial MC, red MV, yellow MA, and black MP).

Table 1: Fatty acid profiles of maca (*Lepidium meyenii* Walp) types, harvested in Cuzco-Peru, from 2018/2 to 2019/1.

Sample	Peak	R. Time	%Area	Symbology	Substance
Commercial (MC)	1	9.87	13.57	16:00	Palmitic acid
	2	12.748	40.1	18:3n-6	Linolenic acid
	3	12.84	39.17	18:1n-9	Oleic acid
	4	13.266	2.86	17:00	Margaric acid
Red (MV)	1	9.87	13.02	16:00	Palmitic acid
	2	12.751	43.35	18:3n-6	Linoleic acid
	3	12.842	40.79	18:3n-1	Oleic acid
	4	13.265	1.89	17:00	Margaric acid
Yellow (MA)	1	9.87	42.97	16:00	Palmitic acid
	2	12.751	16.09	18:3n-6	Linolenic acid
	3	12.857	12.53	18:3n-3	α -Linolenic acid
	4	12.95	7.22		(trans)-2-nonadecene
	5	13.268	21.2		Heptadecan acid, 16-methyl-, methyl ester
Black (MP)	1	9.87	27.63	16:00	Palmitic acid
	2	12.751	29.56	18:3n-6	Linolenic acid
	3	12.856	25.95	22:2n-6	8,11,14-Docosatrienoic acid, methyl ester
	4	12.937	10.05	22:1n-9	Erucic acid
	5	13.272	6.81		Heptadecan acid, 16-methyl-, methyl ester

Source: own author (2020).

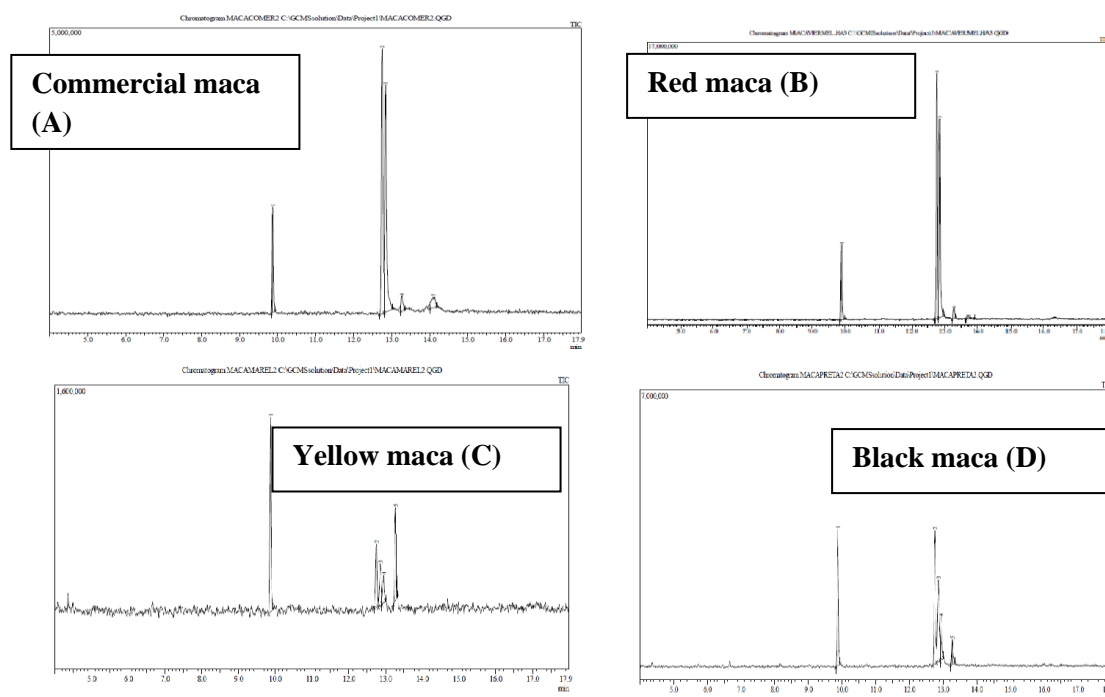
High unsaturated fatty acid content is a desirable attribute of food products since consumption of unsaturated fatty acids is associated with low levels of high cholesterol in the blood. Unsaturated fatty acids therefore play important roles in metabolism and have significant nutritional and industrial applications.

Variation in fatty acid profiles was detected amongst the analyzed samples, with the unsaturated fatty acids comprising the bulk of total fatty acid content. In comparison, 40.79% oleic acid is found in red maca Palmitic acid was detected in all samples, with the highest percentage in the yellow variety (42.97%), and Margic acid was found in both commercial samples (2.86%) and red variety samples (1.89%). Black maca (MP) displayed a distinct fatty

acid profile and contained 25.95% 8,11,14-docosatrienoic acid, methyl ester, and 10.05% erucic acid.

Esparza et al. (2015) demonstrated that maca (*Lepidium meyenii*) possesses high levels of fatty acids, mainly including linolenic, oleic, and linoleic acids, thus corroborating results of our study. In addition, Dini et. al., (1994) and Dufour & Loonis, (2005) studied the chemical composition and regional and stereo selective oxidation of serum albumin-linked linoleic acid in maca (*Lepidium meyenii*) and found fatty acid profiles in maca similar to those in our study. A lack of unsaturated fatty acids leads to skin problems like alopecia, a peeling epidermis, and eczema (Yang & Kallio, 2001). Additionally, according to Cozza & Costa, (2000), high blood cholesterol levels can lead to coronary heart disease and can be prevented through lower consumption of saturated fatty acids and increased consumption of polyunsaturated fatty acids (PUFAs). Figure 1 shows fatty acid concentrations in maca varieties (commercial MC, red MV, yellow MA, and black MP).

Figure 1: Fatty acid CG/MS profiles of different maca (*Lepidium meyenii* Walp) varieties: commercial maca (A), red maca (B), yellow maca (C), and black maca (D). harvested in Cuzco-Peru, from 2018/2 to 2019/1. Fatty acid concentrations are presented as mg/100 g.



Source: own author (2020)

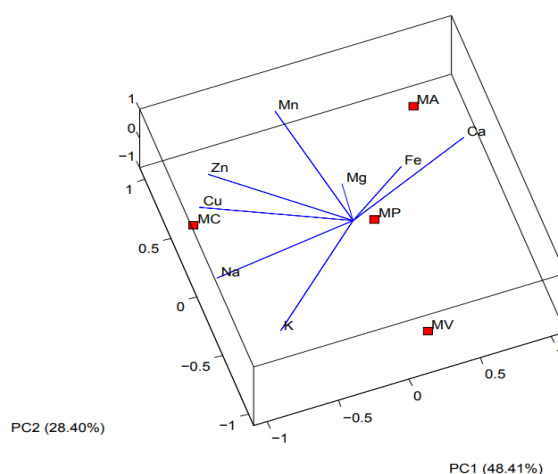
The fatty acids we identified perform several actions in the body and there is growing interest in foods rich in polyunsaturated fatty acids, including linoleic acid, linolenic acid, and

arachidonic acid, which is also known as vitamin F and is necessary for skin growth and protection (Wannes & Marzouk, 2016). The chromatographic peaks shown above in Figure 1, for the fatty acid content, corroborate with the values described in table 1.

3.2. Mineral composition

The highest levels of minerals detected were in commercial maca (MC), which contained 490.65 mg/100 g Ca²⁺ and 1472.65 mg/100 g K⁺, followed by red maca (MV) with 1478.25 mg/100 g K⁺ and 670.25 mg/100 g Ca²⁺, yellow maca (MA) with 875.65 mg / 100 g Ca²⁺ and 1215.25 mg/100 g K⁺, and black maca (MP) with 609.23 mg/100 g Ca²⁺, 1425.32 mg/100 g K⁺, and 45.36 mg/100 g Fe, which is reflected by PCA results presented in Figure 2.

Figure 2: Principal component analysis (PCA) of mineral composition of maca (*Lepidium meyenii* Walp) varieties MA (yellow), MC (commercial), MV (red) and MP (black), harvested in Cuzco-Peru, from 2018/2 to 2019/1.



Source: own author (2020)

Maca types analyzed showed high potassium and calcium levels, according to the observed in Figure 2. Zhang et. (2015) when compared the mineral content of maca (*Lepidium meyenii*) found in Asia and South America and detected average sodium values of 1670 mg/kg. Our work reported levels of Ca²⁺ and K⁺, which are fundamental elements for proper human nutrition. According to Wood and Zheng (1997), in addition to its role in building and

maintaining bones and teeth, calcium has many metabolic roles in cells of other tissues. Higher potassium levels in maca compared to our study were detected by Dini et al., (1994), who found that maca (*Lepidium meyenii*) on average contains 2050 mg/100 g potassium. Vegetables are generally the main mineral sources for humans and mineral availability is closely related to soil content.

Within vegetables, minerals are present in their natural organic complex forms, which are readily usable by the body (Food Ingredients Brazil, 2008). However, the quantity of these minerals is not always sufficient to meet nutritional requirements, requiring supplement consumption.

3.3. Saponin detection and diosgenin bioaccessibility

As presented in Table 2, MC contained the highest levels of saponins at 250.33 mg/100 g for saponins and showed comparable levels of diosgenin (340.56 µg/mL) to MA. Table 2 shows fatty saponin and diosgenin levels in maca varieties (commercial MC, red MV, yellow MA, and black MP).

Table 2: Detection of saponin and diosgenin levels in different types of maca, harvested in Cuzco-Peru, from 2018/2 to 2019/1.

Products	Saponin(mg/100g)	Diosgenin (µg/ml)
Control (MC)	250.33a±2.31	340.56a±1.25
Yellow (MA)	190.36b±10.23	340.25a±1.32
Red (MV)	170.36c±2.33	180.26b±3.25
Black (MP)	206.32b±9.35	30.23c±5.25

Means followed by the same letter in the column do not differ from each other (p <0.05).

Source: own author (2020)

The high levels of diosgenin in all four analyzed maca varieties we studied and demonstrated in Table 2 reflects the importance of maca consumption of this product.

According to Raju, et al. (2009), saponins are a diverse group of glycosidic compounds found naturally in edible and inedible plants that possess a range of health benefits properties and are can be used as drug precursors. Diosgenin, a type of saponin, is a

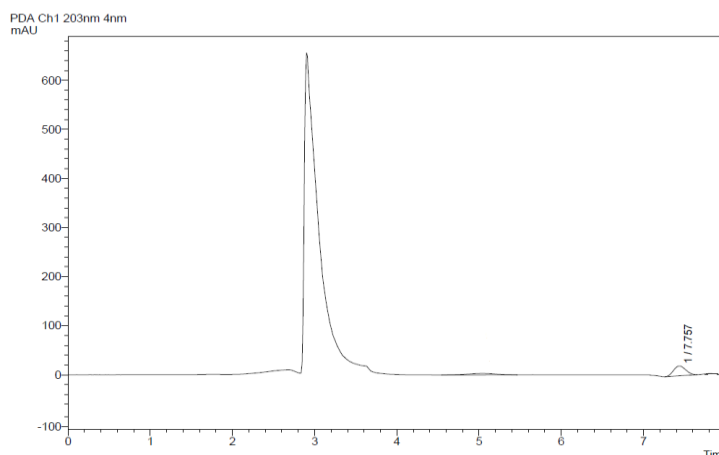
steroidal saponin found in several plant species and is one of the main bioactive constituents of multiple plant species, including yam (*Dioscorea villosa*) and maca (*Lepidium meyenii*) (Raju, et al, 2009 Taylor et al 2000; Zhang et al., 2015). In terms of other types of saponins, MP showed the lowest levels of diosgenin at 30.23 µg/ml. In comparison, Sanchez Mendoza et al. (2016) detected saponin values of 32.35 mg/g in ingá seeds while Desai et. al. (2015) detected mean diosgenin concentrations of 42.5623 µg/mL in *Solanun* extracts.

Diosgenin can be chemically converted to various human hormones, such as progesterone, aldosterone, cortisol, and estrogen, through various enzymatic reactions (Nique et. al., 2012; Hsu, K., et al, 2008; Tucci M., et al., 2003). Reyna-Villasmil et. al. (2008) reported that consuming a diosgenin dose of 510 mg/day significantly affected CRP concentrations and did not change homocysteine concentrations, unlike consumption of soy, isoflavones, and genistein.

Such an increase in CRP is related to a significant increase in cardiovascular disease risk and may be implicated in the development of coronary heart disease and venous thrombosis. In addition to improving hormone levels, diosgenin may inhibit certain inflammatory processes and carcinogenesis. In addition, multiple studies have shown diosgenin modulation of processes involving cyclooxygenase (COX) and lipoxygenase (LOX) (Molaic et al. (2001); Leger et. al., (2004); Chen et al. (2006); Furstenberger et.al, (2006)). These two enzyme types are responsible for eicosanoid biosynthesis. The enzyme COX-2 participates in the conversion of arachidonic acid (AA) into prostaglandins (PG) and thromboxane (TX) and the involvement of COX-2 in inflammatory processes and carcinogenesis is well known.

Studies have also shown that diosgenin inhibits COX-2 enzyme activity and expression in osteosarcoma 1547 cells. Additionally, Li et. al. (2005) and Chen et. al. (2011) reported that diosgenin treatment leads to cell cycle arrest and apoptosis due to activation of cPLA2 and the overexpression of COX-2 in erythroleukemic cells. In addition, the effect of diosgenin on breast cancer cells was evaluated in an electrochemical study and revealed that diosgenin effectively inhibits viability and proliferation. Figure 3 shows chromatogram for diosgenin detection in maca varieties (commercial MC, red MV, yellow MA, and black MP).

Figure 3: Chromatogram for diosgenin detection via HPLC, harvested in Cuzco-Peru, from 2018/2 to 2019/1.



Source: own author (2020)

The detection of diosgenin showed in Figure 3, this important because the diosgenin, can be chemically converted to various human hormones, such as progesterone, aldosterone, cortisol, and estrogen, through various enzymatic reactions (Nique et. al., 2012; Hsu, K., et al, 2008; Tucci M., et al., 2003). Reyna-Villasmil et. al. (2008) reported that consuming a diosgenin dose of 510 mg/day significantly affected CRP concentrations and did not change homocysteine concentrations, unlike consumption of soy, isoflavones, and genistein.

4. Conclusion

Maca was a good source of minerals, especially Ca^{2+} and K^{+} , and the unsaturated fatty acids, linoleic and linolenic acid.

High levels of saponins and diosgenins were detected in the different types of maca analyzed, with the highest percentages found in commercial yellow and red maca varieties, respectively.

These results emphasize the importance of maca consumption on human metabolism. However, future works studying the potential of maca with food source and diosgenin are necessary, in order to discover all the nutritional potential that this product may have.

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Conflict of Interest

The authors declare that there is no conflict of interest

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