How to Cite:
Gutiérrez, M. A., Chávez, B. H., Gallegos, R. M. Z., & Borja, S. F. (2022). Characterization of natural extracts obtained by supercritical fluids (SCF) from Mashua tubers (Tropaeolum Tuberosum) grown in Ecuador. *International Journal of Health Sciences*, *6*(S8), 1286–1293. https://doi.org/10.53730/ijhs.v6nS8.9968

Characterization of natural extracts obtained by supercritical fluids (SCF) from Mashua tubers (Tropaeolum Tuberosum) grown in Ecuador

Marco Arellano Gutiérrez
State University of Bolivar, Faculty of Agricultural Sciences, Agroindustry and Agronomy Careers, Biotechnological Research and Development Center, Guaranda EC020150, Ecuador

Byron Herrara Chávez*
Animal Production and Industrialization Research Unit, Engineering Faculty, National University of Chimborazo, Riobamba EC060150, Ecuador
*Corresponding author

Ronald Mauricio Zurita Gallegos
Higher Polytechnic School of Chimborazo, Faculty of Public Health, Gastronomy Career, Riobamba EC060150, Ecuador

Sonia Fierro Borja
State University of Bolivar, Faculty of Agricultural Sciences, Agroindustry and Agronomy Careers, Biotechnological Research and Development Center, Guaranda EC020150, Ecuador

***Abstract*---**The mashua (Tropaeolum tuberosum) is a plant native to the central Andes whose cultivation is concentrated in Colombia, Bolivia, Ecuador and Peru between 3500 and 4100 meters of altitude, this tuber is rich in nutrients due to its bioactive properties, generally in Ecuador it is cultivated in association with the oca, and the melloco. Therefore, the purpose of this work was to characterize by chromatography two varieties of white and yellow Mashua. Therefore**,** the supercritical fluid extraction method (FSC) was applied in the two varieties, the volatile compounds of the obtained extracts were characterized by Gas Chromatography coupled to a Mass Spectrometer (GC-MS). After characterization, in general, 16 and 11 major volatile compounds considered to belong to the families or functional groups of ketones, fatty acids, furans, furfurals, alcohols, phyto esters, alkaloids and sterols were identified, some of these compounds considered useful for the prevention of diseases such as cancer, they also have antioxidant properties, hypoglycein agent and in the inhibition of the thyroid gland.

Manuscript submitted: 9 March 2022, Manuscript revised: 18 May 2022, Accepted for publication: 27 June 2022

***Keywords*---**characterization, extracts, SCF, mashua.

Introduction

Mashua (*Tropaeolum tuberosum*) belongs to the Tropaeolaceae family. It is a plant native to the central Andes whose cultivation is concentrated in Colombia, Bolivia, Ecuador and Peru between 3.500 and 4.100 meters of altitude, it produces between 9 and 70 mt/ha. The productivity of the mashua has surpassed that of the potato in two for one since it is very tolerant, it grows in poor soils and without fertilizers, this especially since it is resistant to pests and diseases (Campos et al., 2018; Bonete, 2016). Its cultivation cycle varies between 6 and 9 months, reaching higher yields than other Andean tubers (Pacco, 2015).

Mashua tubers have a high protein content (higher than those of potatoes, oca and olluco), carbohydrates, fiber, ascorbic acid (vitamin C) and calories. They also contain a high concentration of aromatic glucosinolates that, when hydrolyzed, are transformed into isothiocyanates, chemical compounds responsible for giving the typical spicy flavor to tubers. Isothiocyanates are known for their antibiotic, anticancer and diuretic properties, which is why it is widely used in the folk medicine of the Andes (Morillo, 2016; Manrique, 2013).

In Ecuador it is grown in the provinces of Chimborazo, Pichincha, Cotopaxi, Cañar, Bolivar, Imbabura and Carchi. According to the INIAP (National Institute of Agricultural Research) in Ecuador, the mashua is cultivated together with the oca, and the melloco in an associated way (INIAP, 2010). There is a great variability of mashua genotypes in terms of shape and color. The color varies from white to yellow, there are also some red or purple variants, this diversity seems to be correlated with the content of bioactive compounds present (Chirinos et al., 2008).

Chemical Composition of Mashua

This Andean tuber has a high nutritional value and comparatively high water content, ranging from 79 to 94% in fresh or edible matter. The main nutritional contribution of mashua is its high content of carbohydrates, particularly starch, and also of sugars (20%) (Morillo, 2016). The protein content of mashua in fresh matter is high (15%), it also has potential medicinal properties for the pharmaceutical industry (Aruquipa et al, 2017).

Extraction by Supercritical Fluid

Supercritical fluid is called a certain pure substance that is operating in conditions of pressure and temperature above its critical point; in a phase diagram this zone is designated as "supercritical state" and exhibits peculiar properties in the compounds, so that they present characteristics of both liquids and gases, this particularity allows them to have high density, low viscosity and high diffusivity that are properties susceptible to changes either due to the effect of increased or decreased pressure or temperature (Cardona & Orrego, 2009).

Gas Chromatography

It is a method that allows the separation of complex mixtures. But once all the individual components of a problem sample have been separated, detected and quantified, the only data available for the identification of each of them is the retention time of the corresponding chromatographic peaks. This information is not sufficient for an unequivocal identification, especially when analyzing samples with a high number of components (Gutierrez Gaiten, 2013). Considering these antecedents, the objective of this research was to characterize by chromatography two varieties of Mashua cultivated in Ecuador.

Materials and Methods

The research was developed in the facilities of the Research Laboratories of the State University of Bolivar.

Sampling

In the present investigation, we worked with two varieties of mashua: white mashua and yellow mashua, from the parishes: Quizapincha from Ambato and Salinas from Guaranda, respectively.

Study Factors

We worked with two factors; factor A: varieties of tubers and factor B: extraction method (see table 1).

Table 1: Study factors

Factors	Code	Levels
Varieties of tubers	A	a_1: Yellow Mashua a_2: White Mashua
Methods	B	b Supercritical fluid extraction

Each experimental unit is made up of 2000 g, under the design (A*B).

Experiment Management

The preparation of the samples at the different levels of processing: Reception and classification (selecting the tubers that do not show damage), Washing, Peeling (removal of the rind), Deep-frozen (-81°C), Freeze-dried: (conditioning of the freeze-dryer, vacuum pump heating for 20 minutes at -57 °C, and the main drying for 12 hours at a pressure of 0.03 millibars, and the final drying for 36 hours at a pressure of 0,03 millibars), Milled (particle size reduction).

Supercritical Fluid Extraction

The extraction was carried out in a Helix SFE system, which basically consists of a solvent reservoir (CO_2, Linde SA with 99,95% purity), a CO_2 pump module /

control module and a vessel or base module, in each extraction, the container is loaded with 15 g of ground sample to a certain sieve size, it was worked at constant temperature and pressure considering a static time of 30 minutes and a dynamic time of 2 hours, the extractions were carried out at a temperature of 50°C and pressure of 200 Bar. With a volumetric CO_2 flow rate of 1L ± 0,5 L/min, the extract was collected in a 50 mL vial and stored at -10°C, all tests were performed 7 times.

Methodology to Determine Volatile Compounds in Gas Chromatography Coupled to a Mass Spectrometer

The detection of compounds by GC-EM was carried out in an Agilent Technologies equipment (7890A GC system and 5975C inert XL MSD with triple axis detector). An HP-5MS capillary column (30 m × 250 µm 0,25 µm) with phenylmethylpolysiloxane (0,25 µm film thickness) as stationary phase and helium as carrier gas (0,8 mL/min) was used 1µL of derivatized sample was injected in Split mode using the 1:20 ratio. The injection chamber temperature was 250°C. The oven temperature was maintained from 60°C to 80°C with a 5°C/min ramp then increased to 92°C with a 3°C/min ramp for 5 minutes then increased to 165°C at rate of 4 °C/min and finally it is raised to 290°C at a rate of 2°C/min for 2 min; 70°C for 2 minutes and increased to 300°C at 5°C/min with a waiting time of 6 minutes. The compounds were identified by comparison with the mass spectra of the NIST 2011 library. The mass range used was between 40 - 550 amu or 40 - 550 Daltons.

Results and Discussion

Results obtained by gas chromatography coupled to a mass spectrometer (CG-MS) of yellow and white mashua.

Chromatography of Extract Obtained from Yellow Mashua

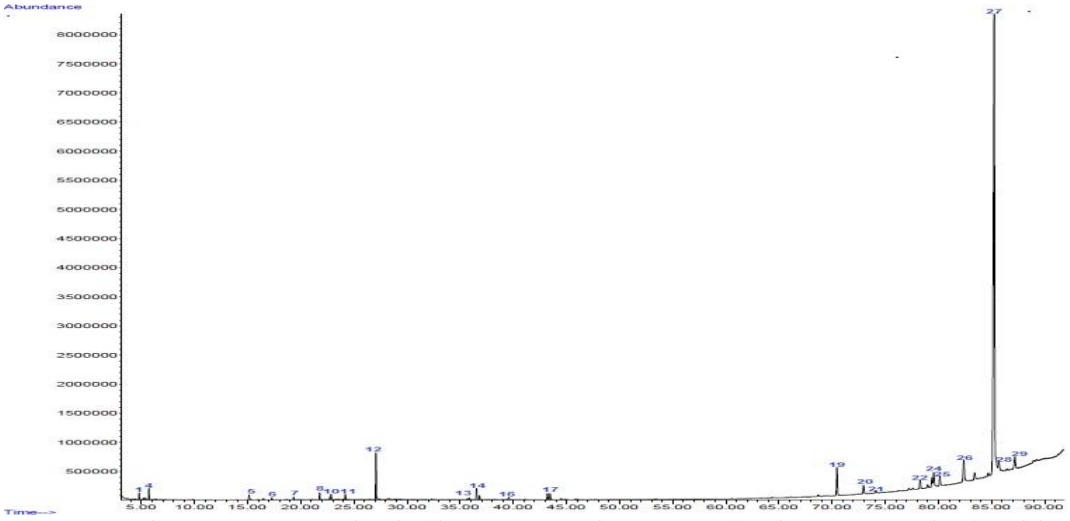

Figure 2: Chromatogram of volatile compounds present in the extract obtained by supercritical fluids Yellow Mashua

Twenty nine volatile compounds were identified in the extract obtained by supercritical fluids from yellow mashua, where 11 major peaks were identified at different retention times and area percentages. In the following order 4-Methoxybenzyl isothiocyanate at 27,070 min with an area of 3,13%; Squalene at min 70,448 with area of 3,05%; Heptacosane at min 72,956 with an area of 1.18% of; Stigmast-5-en-3ol, oleate at 78,271 min with an area of 1,5 %; 1,3-Dioctadecyloxypropane at 79,392 min with 1,11 % of area; 3-Hydroxyspirost-8-en-11-one # al min 79,550 with an area of 1,94 %; α-Tocopherol at min 80,132 with area of 1,513 %; α-Tocopherol at 82,388 min with an area of 3,29 %; γ-Sitosterol at 85,245 min with 73,4 % of area; Spirost-8-en-11-one, 3-hydroxy-, (3β,5α14β,20β,22β,25R)- at 85,687 min with 1,93 % of area; Methyl glycocholate, 3TMS derivative at min 87,195 with an area of 1,37 %.

According to the research carried out by Jimenez et al, (2020), it presents coincidences in five of eleven major compounds found in our work, such as Scualen, γ-Sitosterol, α-Tocopherol, Stigmast-5-en-3ol, oleate, γ -Sitosterol. However, in our study there are 29 compounds between major and minor with different groups such as (ketones, aldehydes, surfurales, phyto esters, pyrans and fatty acids).

Chromatography of Extract Obtained from White Mashua

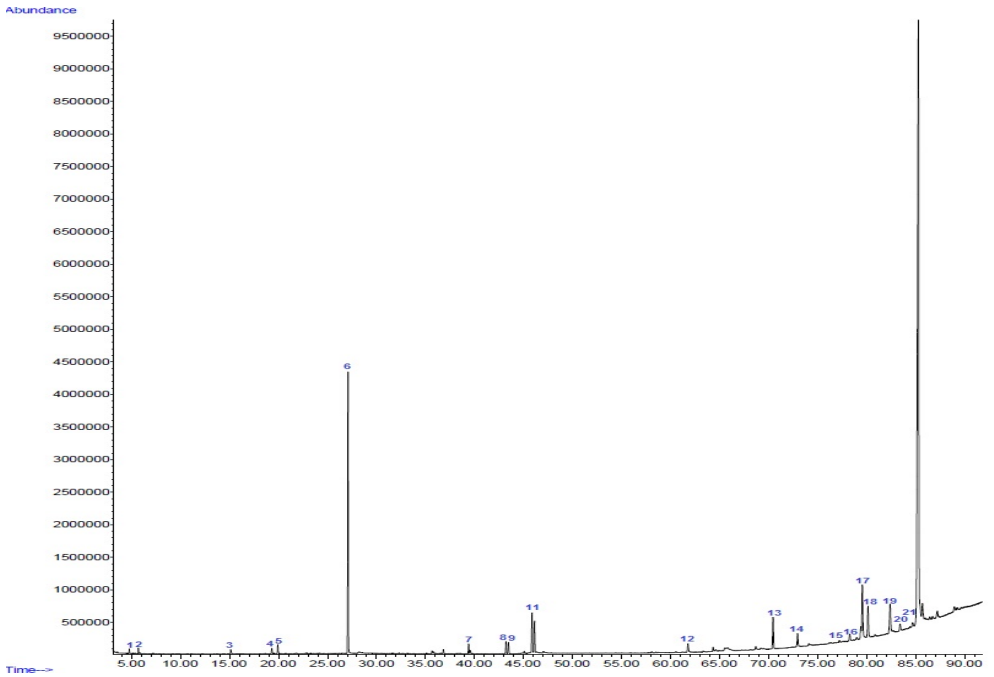

Figure 4: Chromatogram of volatile compounds reported in the extract obtained by supercritical fluids from Mashua Blanca

Twenty one volatile compounds were identified in the extract obtained by supercritical fluids from white mashua, where 17 major peaks were identified at different retention times and area percentages, the volatile compounds were identified using GC/MSD and the ions were compared with the NIST14 library. In

the following order Benzeneacetonitrile, 4-methoxy-al at 19,922 min with an area of 1,27%; 4-Methoxybenzyl isothiocyanate at 27,090 min with 34,53 % of area; Palmitic acid, ethyl ester at 39,398 min with an area of 1,52%; Linoleic acid, methyl ester at min 43,202 min with an area of 2,09 %; Linolenic acid, methyl ester at 43,452 min with an area of 1,98 %; Linoleic acid ethyl ester at 45,855 min with 7,25 % of area; Linolenic acid, ethyl ester at 46,107 min with area of 5,46 %; Octahydrochrom-4,5-dione, 4',8'-epoxy at 61,763 min with 2,04 % of area; Squalene at min 70,440 with an area of 6,13%; Heptacosane at 72,948 min with an area of 2,91 %; Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- at 78,263 min with an area of 1,50 %; 1,3-Dioctadecyloxypropane at 79,392 min with 2,37 % of area; 17-Pentatriacontains at 79,551 min with an area of 12,09 %; α-Tocopherol at 80,127 min with an area of 6,50 %; Campesterol at 82,382 min with an area of 6,77 %; γ-Sitosterol at 83,394 min with an area of 1,70 %; Spirost-8-en-11-one, 3-hydroxy-, (3β,5α14β,20β,22β,25R)- at 84,68 min with an area of 1,30 %.

Six major compounds coincide with the studies carried out by Jimenez et al, (2020), where they present coincidences in γ-Sitosterol, Campesterol, α-Tocopherol, Squalene, Heptacosane and Linoleic acid ethyl ester. However, our study represents a total of 21 compounds belonging to the family of ketones, phyto esters, alcohols, fatty acids, ketones, acetonitriles, aldehydes, etc.

Uses of Some Volatile Compounds Important for Our Health

Some beneficial compounds for the health of human beings identified in the different extracts of the two varieties of mashua are detailed. The identified stigmasterol compound is part of a group of plant sterols or phytoesters, which include campesterol β-sitosterol are compounds that contain provitamin D2. Some research has shown that these compounds may be helpful in preventing cancer-like diseases, including ovarian, prostate, breast, and colon cancer. Also, they have antioxidant properties, hypoglycein agent and in the inhibition of the thyroid gland (Fernández et al., 2003).

There are studies that have shown that scualen has anti-inflammatory properties, a beneficial effect on the skin and plays a role in aging and skin pathologies. Also, it is used as an adjuvant in vaccines and has been tested in other fields such as cancer and dyslipidemia. On the other hand, γ-sitosterol is a potent inhibitor of the C1 complex of the complement component and has shown its potential as a treatment (Rangachari et al., 2011; Gutiérrez et al., 2011).

Conclusions

The use of the Gas Chromatography technique Coupled to a Mass Spectrometer (GC-MS), has allowed the extraction of polar volatile compounds and some of low molecular weight from mashua extracts obtained by FSC, where 16 and 11 major volatile compounds were identified, considered to belong to the families or functional groups of ketones, fatty acids, furans, furfurals, alcohols, phyto esters, alkaloids and sterols.

References

Aruquipa, R., Trigo, R., Bosque, H., Mercado , G., & Condori, J. (2017). El Isaño (Tropaeolum tuberosum) un Cultivo de Consumo y Medicina Tradicional en Huatacana para el Beneficio de la Población Boliviana. RIIARn, 3(2), 146–151. https://doi.org/10.1038/bmt.2010.149.

Bonete. (2016). Estudio de cuatro tubérculos y raíces tuberosas no tradicionales.

Campos, D., Chirinos, R., Galvez Ranilla, L., & Pedreschi, R. (2018). Bioactive Potential of Andean Fruits, Seeds, and Tubers. Advances in Food and Nutrition Research. 84:287-343. doi: 10.1016/bs.afnr.2017.12.005.

Cardona, C. A., & Orrego, C. E. (2009). Avances investigativos en la producción de biocombustibles (Primera Ed). Manizales:. Universidad Nacional de Colombia.

Chirinos, R., Campos, D., Warnier, N., Pedreschi, R., Rees, J.-F., & Larondelle, Y. (2008). Antioxidant properties of mashua (Tropaeolum tuberosum) phenolic extractsagainst oxidative damage using biological in vitro assays. Food Chemistry, 111(1), 98–105.

Fernández, C., Martín, C., Gómez Coronado, D., & Lasunción, M. (2003). Efecto de los fitosteroles sobre la biosíntesis de colesterol y la proliferación en células humanas. [Efecto de los fitoesteroles sobre la biosíntesis y la proliferación del colesterol en células humanas. Clinica e Investigación en Arteriosclerosis, 175-183.

Gutierrez Gaiten , Y. (2013). Estudio Farmacognóstico de Phyllanthus orbicularis HBK, especie endémica de Cuba. (Tesis doctoral, Universidad de La Habana). http:// tesis. repo. sld.cu/ 429/ 1/ GUTI% C3% 89RREZ_ GAIT%C3%89N.pdf

Gutiérrez, G., Miranda, M., Bello, A., Hernández, S., & Pr, M.D. (2011). Caracterización química mediante cromatografía de gases / espectrometría de masas de dos extractos de Phyllanthus orbicularis HBK. Revista Cubana de Farmacia, 45-405-413.

INIAP. (2010). Instituto Nacional de Investigaciòn Agropecuaria.

Jimenez, H. E., Gutierrez, G. Y., Choez, G. I., & Miranda, M. M. (2020). Estudio de actividad farmacognóstica, química y antiinflamatoria de dos variedades de Tropaeolum tuberosum (Ruiz y Pav.) Kuntze (Tropaeolaceae). International Journal of Pharmacy and Chemistry ., 6(4): 41. doi: 10.11648/j.ijpc.20200604.12.

Manrique, I. (2013). Tropaeolum tuberosum Ruíz & Pav . Colección de germoplasma de mashua conservada en el Centro Internacional de la Papa. (CIP).

Morillo, A., Morillo, Y., & Tovar, Y. (2016). Caracterización molecular de cubios (Tropaeolum tuberosum Ruíz y Pavón) en el departamento de Boyacá. Rev. Cienc. 33 (2): 32-42. https:// dx. doi. org/ 10.5897/ AJB2014.14003

Pacco, W. (2015). Evaluación del Efecto del Soleado y la Cocción en la Capacidad Antioxidante del Puré Deshidratado de Mashua (Tropaeolum tuberosum R. et P.). Tesis, Facultad de Ciencias Agrarias, Universidad Nacional del Altiplano, Puno, Perù.. http://repositorio.unap.edu.pe/handle/UNAP/241

Rangachari, B., Veeramuthu, B., & Savarimuthu, I. (2011). Actividad antidiabetica del γ-sitosterol aislado de Lippia nodiflora L. eratas diabéticas inducidas por estreptozotocina. Revista europea de farmacología., 667: 410-418.

Sagita, M. B., Turchan, A., Utomo, B., Fauzi, A. A., & Fauziah, D. (2022). Expression malondialdehyde (MDA) of brain after injury with the extract of

kencur (Kaempferia Galanga L): Experimental study wistar rats. *International Journal of Health & Medical Sciences*, *5*(1), 114-121. https://doi.org/10.21744/ijhms.v5n1.1848

Widana, I.K., Sumetri, N.W., Sutapa, I.K., Suryasa, W. (2021). Anthropometric measures for better cardiovascular and musculoskeletal health. *Computer Applications in Engineering Education*, *29*(3), 550–561. https://doi.org/10.1002/cae.22202