

# Phenolic compounds and *in vitro* antioxidant activity of six accessions of mashua (*Tropaeolum tuberosum* R. & P.) from Puno Region, Peru

Compuestos fenólicos y actividad antioxidante *in vitro* de seis accesiones de mashua (*Tropaeolum tuberosum* R. & P.) de la Región Puno, Perú

<https://doi.org/10.15446/rfnam.v74n3.93020>

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## ABSTRACT

### Keywords:

Antioxidants  
Flavonoids  
HPLC  
Plant tubers  
Polyphenols

Mashua (*Tropaeolum tuberosum* R. & P.) is an Andean crop of high nutritional value and medicinal properties, which presents a great diversity in morphology and color. The aim of the study was to evaluate the content of phenolic compounds and *in vitro* antioxidant activity of the most economically important mashua accessions in the Puno Region, Peru. Six accessions of mashua (three purple-colored and three yellow-colored) were evaluated. The content of total polyphenols, total flavonoids and identification of phenolic compounds was determined by the Folin-Ciocalteu assay, aluminum chloride colorimetric method and HPLC-DAD, respectively. *In vitro* antioxidant activity was evaluated using the FRAP and DPPH assays. In general, the purple-colored mashua had a significantly higher content of total polyphenols, total flavonoids, and *in vitro* antioxidant activity compared to the yellow-colored mashua; being the Tt-23 accession purple-colored (peel/pulp, purple/purple), which presented a significantly higher content of phenolic compounds and *in vitro* antioxidant activity compared to the other accessions evaluated ( $P < 0.05$ ). Furthermore, a significant correlation was observed between FRAP and DPPH activities with the total content of polyphenols and flavonoids ( $P < 0.01$ ), as well as between FRAP activity and the caffeic acid and rutin levels ( $P < 0.05$ ). These results suggest that purple-colored mashua, particularly the Tt-23 accession (peel/pulp, purple/purple), has better nutraceutical and antioxidant properties due to its higher content of phenolic compounds.


## RESUMEN


### Palabras clave:

Antioxidantes  
Flavonoides  
HPLC  
Tubérculos vegetales  
Polifenoles

Mashua (*Tropaeolum tuberosum* R. & P.) es un cultivo andino de alto valor nutricional y propiedades medicinales, que presenta una gran diversidad en morfología y color. El objetivo del estudio fue evaluar el contenido de compuestos fenólicos y la actividad antioxidante *in vitro* de las accesiones de mashua de mayor importancia económica en la Región Puno, Perú. Se evaluaron seis accesiones de mashua (tres de color púrpura y tres de color amarillo). El contenido de polifenoles totales, flavonoides totales e identificación de compuestos fenólicos se determinó mediante el ensayo de Folin-Ciocalteu, método colorimétrico de cloruro de aluminio y HPLC-DAD, respectivamente. La actividad antioxidante *in vitro* se evaluó mediante los ensayos FRAP y DPPH. En general, la mashuas de color púrpura presentaron un contenido significativamente mayor de polifenoles totales, flavonoides totales, y actividad antioxidante *in vitro* en comparación con las mashua de color amarillo; siendo la accesión Tt-23 de color púrpura (piel/pulpa, púrpura/púrpura), la que presentó un contenido significativamente mayor de compuestos fenólicos y actividad antioxidante *in vitro* en comparación con las otras accesiones evaluadas ( $P < 0,05$ ). Asimismo, se observó una correlación significativa entre las actividades de FRAP y DPPH con el contenido de polifenoles y flavonoides totales ( $P < 0,01$ ), así como entre la actividad de FRAP y los niveles de ácido cafeico y rutina ( $P < 0,05$ ). Estos resultados sugieren que las mashua de color púrpura, particularmente la accesión Tt-23 (piel/pulpa, púrpura/púrpura), presenta mejores propiedades nutraceuticas y antioxidantes debido a su mayor contenido de compuestos fenólicos.

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Due to the climate change, it is necessary to search for crops resistant to harsh climates, pests and poor soils that can replace popular crops (Zhang *et al.*, 2018). The search for new plants with antioxidant compounds has increased considerably during the last 5 years (Pisoschi *et al.*, 2016), mainly due to the ability of antioxidants to neutralize free radicals that help prevent cardiovascular and cerebrovascular diseases as well as cancer (Gul *et al.*, 2016). Antioxidants can also prevent atherosclerosis, arthritis, diabetes, and other diseases (Zhang *et al.*, 2015). In the food industry, antioxidants are used to reduce the rate of oxidation of the products and thus, extend their shelf life (Xu *et al.*, 2017). Peru has a high biodiversity of food and medicinal plants with nutraceutical and antioxidant potential, among which are Andean tubers as mashua (*Tropaeolum tuberosum* R. & P.) (Campos *et al.*, 2018; Pacheco *et al.*, 2020). This tuber is a perennial herbaceous plant native to the Andean region with a high nutraceutical potential, which grows between 2,800 and 4,000 masl. Its spread and distribution includes Colombia, Ecuador, Peru, Argentina and Bolivia (Roca *et al.*, 2007; Valle-Parra *et al.*, 2018; Choquechambi *et al.*, 2019; Apaza *et al.*, 2020). In the Andean region, Peru and Bolivia represent the largest planting areas, which is generally grown in association with other tubers such as oca (*Oxalis tuberosa*), ulluco (*Ullucus tuberosus*) and potato (*Solanum tuberosum*) (Manrique *et al.*, 2014). Mashua has a high diversity in morphology and color, which ranges from beige to dark purple. In Peru, more than 100 accessions have been recognized due to their variability in morphology and color, which would be correlated with their levels of phenolic compounds (Campos *et al.*, 2018). Economically, it is the less important Andean tubers; however, it contains phenolic compounds with high antioxidant activity (Campos *et al.*, 2006). Previous studies show that mashua contains glucosinolates (Martin and Higuera, 2016; Villacrés *et al.*, 2016), phenolics and high antioxidant activity (Chirinos *et al.*, 2015). Within the Andean tubers such as potatoes (*Solanum* sp.), oca (*O. tuberosa*) and ulluco (*U. tuberosum*), mashua has the highest antioxidant activity (Campos *et al.*, 2006). Furthermore, mashua has high resistance to pests and plant diseases, helps prevent soil erosion, adapts to cold temperatures and poor soils, has medicinal properties and can be used as

a bioinsecticide. It is used in ethnomedicine to relieve kidney, liver, and prostate disorders, obtaining favorable results due to its bioactive compounds (Grau *et al.*, 2003).

In Peru, Puno Region is the main producer of mashua followed by the Cusco and Ayacucho Regions. It has a planting area of 4,828 ha that produces only 7,368 t year<sup>-1</sup>, compared to the potato production that is 742,924 t year<sup>-1</sup> in the same region (Ministerio de Agricultura y Riego, 2018). This low production is due to its minimal demand since it has a bitter taste because of the presence of its glucosinolates (Martin and Higuera, 2016). Despite the fact that its planting area is less than those other Andean tubers, its cultivation is still important, since it is part of the food security of thousands of peasant families in the Andes through self-consumption or generation of income from the sale of this product (Apaza *et al.*, 2020). Several studies recommend using it as a nutraceutical product or in the food preservation industry (Campos *et al.*, 2006; Chirinos *et al.*, 2007; Chirinos *et al.*, 2008; Chirinos *et al.*, 2015). In the Puno Region, one of the provinces with the highest production of mashua is Yunguyo. Therefore, the aim of this study was to characterize the physicochemical and antioxidant properties of six accessions of mashua (*T. tuberosum* R. & P.) of greatest economic importance in the province of Yunguyo (Puno Region, Peru), which vary according to the shape and color.

## MATERIALS AND METHODS

### Plant material

Six accessions of mashua (*T. tuberosum* R. & P.) were collected in the Yunguyo district, Yunguyo Province, Puno Region, Peru (16°14'39"S, 69°05'34"W). Yunguyo is one of the 13 provinces of the Puno Region, it has an altitude of 3,826 m, an average temperature of 8 °C, a maximum temperature of 17.3 °C and a minimum temperature of -1.3 °C. Three purple-colored mashua: Tt-03 (peel/pulp, purple/purple), Tt-23 (peel/pulp, purple/purple) and Tt-25 (peel/pulp, purple/purple); and three yellow-colored mashua Tt-02 (peel/pulp, yellow/yellow), Tt-11 (peel/pulp, yellow/yellow) and Tt-19 (peel/pulp, yellow/yellow), were provided by the National Institute of Agricultural Innovation ILLPA-Puno of Peru and numbered using the prefix Tt (*T. tuberosum*) (Figure 1). Approximately five units of each accession were



**Figure 1.** Accessions of mashua (*T. tuberosum* R. & P.) from Yunguyo Province, Puno Region, Peru.

disinfected by submersion in 5% sodium hypochlorite for 15 min, then cut to 2.5 mm in thickness and lyophilized for 7.5 h with a minimum temperature of  $-40\text{ }^{\circ}\text{C}$  and 13.33 Pa by a freeze dryer device (Stellar, Millrock Technology, NY, USA). Subsequently, grinding process was performed in a rotor mill (mesh 0.08 mm) and the lyophilized powder was stored at  $-20\text{ }^{\circ}\text{C}$  until the extraction and purification of phenolic compounds took place.

#### Sample preparation

The extraction of phenolic compounds was performed according to Chirinos *et al.* (2007), with minor modifications. Briefly, 5 g of the lyophilized powder from each accession was weighed using an analytical balance (Sartorius Extend Scale, ED224S) and homogenized for 20 min with methanol ( $\text{CH}_3\text{OH}$ ) (Sigma-Aldrich, USA), acetone ( $\text{C}_3\text{H}_6\text{O}$ ) (Sigma-Aldrich, USA), and destilated water (45:45:10), then acidified with 5 drops of 1% chloride acid (HCl) (Sigma-Aldrich, USA). The purification of phenolic compounds was carried out by solid phase separation in columns RP-18 (Lichrolut, Germany) of 60 mL. The column was conditioned with 60 mL of acidified methanol and 50 mL of acidified water, pH 2. Then, 60 mL of the sample was added, the column was washed with 40 mL of acidified water, and the elution was performed with 40 mL of acidified methanol, all the solvent was removed under vacuum on a rotary evaporator (Selecta, Spain) at  $38\text{ }^{\circ}\text{C}$  for 30 min. The purified solid residue was diluted in methanol ( $10\text{ mg mL}^{-1}$ ) and kept at  $-20\text{ }^{\circ}\text{C}$  in a freezer (Thermo Scientific, USA) until use.

#### Total polyphenols

The content of total polyphenols was determined according to Herrera-Calderon *et al.* (2016). Briefly, 0.1 mL of sample was mixed with 1 mL of 10% Folin-Ciocalteu reagent for 5 min at  $25\text{ }^{\circ}\text{C}$ , then 1 mL of 5% sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) (Sigma-Aldrich, USA) was added and the mixture was placed in a water bath at  $45\text{ }^{\circ}\text{C}$  for 30 min. Absorbance was read by means of a spectrophotometer (Pharo 300, Spectroquant, USA) at 725 nm. The results were expressed in mg gallic acid equivalent  $100\text{ g}^{-1}$  fresh weight (mg GAE  $100\text{ g}^{-1}$  FW).

#### Total flavonoids

Total flavonoid content was performed according to Wolfe *et al.* (2008). To 0.250 mL of sample, 1250 mL of 5% sodium nitrite ( $\text{NaNO}_2$ ) (Sigma-Aldrich, USA) was added and it was left to react for 5 min, then 0.150 mL of aluminum chloride ( $\text{AlCl}_3$ ) was added, and the mixture could stand for 5 min. Finally, 0.5 mL of 1 M sodium hydroxide (NaOH) was added to the mixture and it was left in contact for 15 min. The reading was performed at 510 nm by a spectrophotometer (Pharo 300, Spectroquant, USA). The results were expressed in mg catechin equivalent  $100\text{ g}^{-1}$  fresh weight (mg CE  $100\text{ g}^{-1}$  FW).

#### Antioxidant capacity by ferric reducing antioxidant power (FRAP)

Antioxidant activity evaluated by the FRAP assay was performed according to the methodology proposed by

Szollosi and Varga (2002). Briefly, to 20  $\mu\text{L}$  of sample, 1 mL of distilled water and 1 mL of FRAP reagent (Sigma-Aldrich, USA) were added, the mixture was placed in a water bath at 37 °C and allowed to react for 15 min. The reading was made using a spectrophotometer (Pharo 300, Spectroquant, USA) at 593 nm. A standard curve was prepared using different concentrations of  $\text{Fe}^{2+}$  ranged from 15 to 75 mM. The results were expressed in  $\text{mM Fe}^{2+} 100 \text{ g}^{-1}$  fresh weight ( $\text{mM Fe}^{2+} 100 \text{ g}^{-1}$  FW).

#### Antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

Antioxidant activity evaluated by the DPPH radical scavenging assay was determined through the method developed by Brand-Williams *et al.* (1995), with minor modifications. It was performed using different concentrations of sample, which were placed in test tubes containing 1 mL of 0.1 M Acetate buffer pH 6.0, 1.5 mL methanol and 0.5 mL of 0.1 mM DPPH, then the mixture was stirred at 2500 rpm for 1 min and incubated at 37 °C for 30 min. Absorbance was read by a spectrophotometer (Pharo 300, Spectroquant, USA) at 517 nm. The results were expressed as  $\mu\text{M}$  Trolox equivalent antioxidant capacity 100  $\text{g}^{-1}$  fresh weight ( $\mu\text{M TEAC } 100 \text{ g}^{-1}$  FW).

#### Identification of phenolics compounds by HPLC-DAD

HPLC analyses of caffeic acid, rutin, chlorogenic acid, quercetin, apigenin and kaempferol were performed according to Chirinos *et al.* (2008), with minor modifications. All phenolic compound standards were obtained from Sigma-Aldrich, USA. Briefly, a VWR HITACHI Chromaster 600 HPLC with a diode array detector (HPLC-DAD 300), autosampler and a reversed phase C18 column (5  $\mu\text{m}$  particle size, i.d. 4.6x250 mm) was used. The mobile phase consisted of 0.1% acetic acid solution (A) and 100% acetonitrile (B). The gradient profile was from 10 to 90% B from 0 to the desired gradient time (28, 39 and 55 min) at a flow rate of 0.5  $\text{mL min}^{-1}$ . Detection was performed at 272 and 414 nm using a photodiode array detector. Calibration curves were made in triplicate using five different concentrations (10, 20, 30, 40, 60  $\mu\text{g mL}^{-1}$ ) for all phenolic compounds evaluated (Seal 2016). Data were processed using OpenLAB CDS software (Agilent Technologies, USA).

#### Statistical analysis

All the assays were carried out in triplicate. The results were expressed as mean  $\pm$  standard deviation (SD), and analyzed using SPSS software for Windows version 26.0 (SPSS, Inc., Chicago, IL, USA). The means were compared by one-way ANOVA followed by Tukey's multiple comparison test, at a significance level of  $P < 0.05$ . Statistical correlation among different variables was performed using the Pearson coefficient ( $r$ ) and results were statistically significant when  $P < 0.05$ .

## RESULTS AND DISCUSSION

#### Total polyphenols

Table 1 shows the total content of polyphenols and flavonoids, DPPH radical scavenging and FRAP activity of all samples evaluated. The content of total polyphenols ranged from 75.08 to 221.07  $\text{mg GAE } 100 \text{ g}^{-1}$  FW. All three purple-colored mashua showed significantly higher levels of total polyphenols compared to the three yellow-colored mashua ( $P < 0.05$ ). Within the group of purple-colored mashua, the Tt-23 accession presented significantly higher levels of total polyphenols compared to the Tt-03 and Tt-25 accessions ( $220.83 \pm 0.42$ ,  $172.62 \pm 0.76$  and  $173.55 \pm 0.10$ , respectively).

These results agreed with a previous study (Chirinos *et al.*, 2006) in three purple-colored mashua, which showed a total polyphenol content that ranged from 174.9 to 374.4  $\text{mg GAE } 100 \text{ g}^{-1}$  FW. In another study (Campos *et al.*, 2006), the content of total polyphenols was evaluated in four Andean tubers species. In the native potato (*Solanum* sp.), oca (*O. tuberosa* Molina) and ulluco (*U. tuberosus* Caldas), the content of total polyphenols was ranged from 64 to 232  $\text{mg chlorogenic acid equivalent } 100 \text{ g}^{-1}$  FW, 71 to 131  $\text{mg chlorogenic acid equivalent } 100 \text{ g}^{-1}$  FW, respectively; while in mashua, in the case of the 11 genotypes evaluated, the total polyphenol levels ranged from 92 to 337  $\text{mg chlorogenic acid equivalent } 100 \text{ g}^{-1}$  FW. Studies carried out on different foods show that content greater than 100  $\text{mg GAE } 100 \text{ g}^{-1}$  is recognized as a high content of polyphenols (Ovaskainen *et al.*, 2008). These results showed that the Tt-23 accession purple-colored had a high content of total polyphenols, even higher than other Andean tubers previously evaluated (Campos *et al.*, 2006).

**Table 1.** Total content of polyphenols and flavonoids, DPPH radical scavenging and FRAP activity of six accessions of mashua (*T. tuberosum* R. & P.) from the Puno Region, Peru.

Accessions	Total polyphenols (mg GAE 100 g <sup>-1</sup> FW)	Total flavonoids (mg CE 100 g <sup>-1</sup> FW)	FRAP activity (mM Fe <sup>2+</sup> 100 g <sup>-1</sup> FW)	DPPH activity (μM TEAC 100 g <sup>-1</sup> FW)
Tt-02	90.06±1.83 c	3.10±0.48 f	985.63±4.62 d	43.00±1.44 c
Tt-03	172.62±0.76 b	77.30±0.20 b	1242.52±16.67 b	60.84±1.53 b
Tt-11	90.83±1.20 c	4.23±0.51 e	390.68±14.30 f	28.35±0.65 e
Tt-19	77.48±2.35 d	8.00±0.35 d	460.78±4.20 e	35.26±1.08 d
Tt-23	220.83±0.42 a	79.66±0.19 a	2299.03±25.46 a	68.25±1.80 a
Tt-25	173.55±0.10 b	10.03±0.26 c	1055.53±4.66 c	60.28±1.10 b

Values (mean±SD) in the same column with different letters (a–f) are significantly different (One-way ANOVA with Tukey's post hoc test,  $P<0.05$ ). GAE: Gallic acid equivalent; CE: Catechin equivalent; TEAC: Trolox equivalent antioxidant capacity; FW: fresh weight.

### Total flavonoids

In the mashua accessions evaluated, the total flavonoid levels were ranged from 2.54 to 79.77 mg CE 100 g<sup>-1</sup> FW. As shown in Table 1, the purple-colored mashua (Tt-23 and Tt-03) presented significantly higher content of total flavonoids (79.66±0.19 and 77.30±0.20 mg CE 100 g<sup>-1</sup>, respectively), approximately 8 times higher compared to the other mashua accessions evaluated ( $P<0.05$ ). There are no reports on total flavonoid levels in mashua, however; some flavonoids such as flavan 3-ols, anthocyanins, flavones, flavonols and flavanones have been detected by high-performance liquid chromatography (Chirinos *et al.*, 2008; Pacheco *et al.*, 2019).

### *In vitro* antioxidant activity by FRAP and DPPH radical scavenging assays

In the different mashua accessions evaluated, the FRAP activity ranged 376.89 to 2327.18 mM Fe<sup>2+</sup> 100 g<sup>-1</sup> FW, while the DPPH radical scavenging activity varied from 376.89 to 68.25±1.80 μM TEAC 100 g<sup>-1</sup> FW. As shown in Table 1, all the purple-colored mashua presented significantly higher FRAP and DPPH radical scavenging activity compared to all yellow-colored mashua ( $P<0.05$ ), being again the Tt-23 accession purple-colored, the one that presented the highest FRAP and DPPH radical scavenging activity compared to the other accessions ( $P<0.05$ ).

Mashua accessions present a high diversity in their morphology and color, ranging from beige to purple. Previous studies have shown that purple-colored mashua

have 8 to 10 times more antioxidant activity than yellow-colored mashua. This increase in the *in vitro* antioxidant activity is due partially to its high anthocyanin content (Campos *et al.*, 2006; Chirinos *et al.*, 2006; Chirinos *et al.*, 2008). Chirinos *et al.* (2006) reported that mashua anthocyanins contributed to the total antioxidant activity in only one of the three purple-colored mashua, which allows to hypothesize that other phenolic compounds could participate in its antioxidant activity. Some studies have evaluated the *in vitro* antioxidant activity in different genotypes of mashua using the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and oxygen radical absorbance capacity (ORAC) assays (Campos *et al.*, 2006; Chirinos *et al.*, 2006; Chirinos *et al.*, 2008). A study in 11 pigmented genotypes of mashua (*T. tuberosum* R. & P.) reported that the *in vitro* antioxidant activity evaluated by the ABTS method ranged between 3.82 and 39.15 μmol Trolox equivalent g<sup>-1</sup> FW, finding that the purple-colored DP-02-24, ARB-5241 and ARV-5366 genotypes showed the highest antioxidant activity (Campos *et al.*, 2006). Another study in three purple-colored mashua showed an *in vitro* antioxidant capacity evaluated by the ABTS method, which ranged from 16.2 to 45.7 μmol Trolox equivalent g<sup>-1</sup> FW (Chirinos *et al.*, 2006). Furthermore, when the antioxidant activity was evaluated by the ORAC method in two purple-colored mashua, the values ranged from 221 to 359 μmol Trolox equivalents g<sup>-1</sup> dry matter. These results agree with the present study, which among the different accessions of mashua evaluated, all purple-colored mashua (peel/pulp, purple/purple) showed an increased for the for the *in vitro* antioxidant activity.

### Identification of phenolic compounds by HPLC-DAD

Table 2 shows the phenolic compounds analysis of the six accessions. The HPLC-DAD analysis showed detectable values for caffeic acid, rutin, chlorogenic acid

and quercetin. As shown in Table 2, the highest levels of phenolic compounds were observed for caffeic acid, which ranged from 4.77 to 267.25 mg 100 g<sup>-1</sup>, lower levels were detected for the other phenolic compounds.

**Table 2.** Phenolic compounds measured by HPLC-DAD in six accessions of mashua (*T. tuberosum* R. & P.) from the Puno Region, Peru

Accessions	Cafeic acid	Rutin	Chlorogenic acid	Quercetin
	(mg 100 g <sup>-1</sup> )			
Tt-02	251.01±15.74 a	5.58±0.40 c	7.76±0.05 e	2.53±0.04 c
Tt-03	39.54±3.38 c	7.30±0.44 b	25.43±0.36 a	7.66±0.12 a
Tt-11	8.44±0.37 d	7.89±0.16 b	18.55±0.08 b	3.89±0.05 b
Tt-19	5.30±0.48 d	6.75±1.09 b,c	8.73±0.22 d	0.11±0.01 f
Tt- 23	169.19±1.12 b	9.81±0.02 a	5.96±0.15 f	1.69±0.02 e
Tt- 25	8.97±0.19 d	5.82±0.14 c	12.37±0.13 c	2.11±0.04 d

Values (mean±SD) in the same column with different letters (a–e) are significantly different (One-way ANOVA with Tukey's post hoc test,  $P<0.05$ ).

A previous study, in 3 genotypes of colored mashua from Peru, the presence of gallic acid, galocatechin, epigallocatechin, procyanidin B2 and derivatives of epigallocatechin, rutin and/or derivatives of myricetin and different derivatives of hydroxycinnamic and hydroxybenzoic acid were identified in these accesions (Chirinos *et al.*, 2008). Furthermore, Pacheco *et al.* (2019) in a mashua from Ecuador reported the presence of flavonols and flavan 3-ols of 68.8 and 31.2%, respectively. Among the flavanols, (+)-galocatechin, (-)-epigallocatechin and (-)-epicatechin were identified, being the latter the most abundant (9.22 µg g<sup>-1</sup> dry matter). Isorhamnetin 3-rutinoside, quercetin 3-rutinoside, and quercetin and myricetin derivatives were found. Furthermore, in purple-colored mashua, the presence of 11 anthocyanins such as delphinidin 3-glucoside-5-acetylramnoside, delphinidin

3-glucoside-5-rhamnoside, delphinidin 3-sophoroside-5-rhamnoside, delphinidin 3-glucoside, cyanidin 3-sophoroside, cyanidin 3-sophoroside-5-rhamnoside, cyaniding 3-glucoside, cyanidin 3-rutinoside, pelargonidin 3-sophoroside and pelargonidin 3-sophoroside-5-rhamnoside were identified; of which the first 2 pigments were found in a higher concentration. This anthocyanin-rich fraction was significantly correlated with the *in vitro* antioxidant activity evaluated by an ABTS assay and the content of phenolic compounds ( $r=0.6379$  and  $r=0.9873$ , respectively) (Chirinos *et al.*, 2006).

Other phenolic compounds have not been evaluated in mashua. The present study found an association between *in vitro* antioxidant activity and the content of phenolic compounds other than anthocyanins. Table 3 exposes the correlation between *in vitro* antioxidant activity and

**Table 3.** Correlation between *in vitro* antioxidant activity and levels of total polyphenols, total flavonoids and phenolic compounds identified by HPLC-DAD of six accessions of mashua (*T. tuberosum* R. & P.) from the Puno Region, Peru.

	Correlation (r)					
	Total polyphenols	Total flavonoids	Caffeic acid	Rutin	Chlorogenic acid	Quercetin
FRAP activity	0.883**	0.797**	0.508*	0.570*	-0.280	0.046
DPPH radical scavenging activity	0.945**	0.760**	0.227	0.296	-0.034	0.211

\*  $P<0.05$ , \*\*  $P<0.01$

levels of total polyphenols, total flavonoids and phenolic compounds identified. A significant correlation was observed between the FRAP activity and the total content of polyphenols and flavonoids ( $P < 0.01$ ), as well as with caffeic acid and rutin levels ( $P < 0.05$ ), while the DPPH activity was correlated with the total content of polyphenols and flavonoids ( $P < 0.01$ ) (Table 3). Furthermore, a significant correlation was observed between FRAP and DPPH radical scavenging activity ( $r = 0.873$ ,  $P < 0.01$ , data not shown). These findings show that phenolic compounds other than anthocyanins could also contribute to the *in vitro* antioxidant activity presented by different accessions of mashua.

## CONCLUSIONS

The present study shows that among the six mashua accessions evaluated in the Puno Region, Peru; the purple-colored mashua present a high content of total polyphenols and flavonoids, as well as a high *in vitro* antioxidant activity; the latter significantly correlated with the levels of total polyphenols and flavonoids, as well as phenolic compounds such as caffeic acid and rutin. These results highlight that the high antioxidant activity of this Andean tuber could be explained by bioactive compounds other than anthocyanins, which could be used as a nutraceutical in food and beverages to minimize the risk of diseases caused by oxidative stress, liver and kidney diseases, as well as urinary and prostate disorders.

## ACKNOWLEDGMENTS

This study was funded by the National Program for Agricultural Innovation (PNIA) and Universidad Científica del Sur, Lima, Peru (Contract N°005-2016-INIA-PNIA/UPMSI/IE).

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