Investigation note

Morphometric analysis of flour, starch and protein of *Tropaeolum tuberosum* Ruiz and Pavón

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Abstract

Mashua (*Tropaeolum tuberosum* Ruiz and Pavón) is an edible tuber and was domesticated in the central Region of the Andes; it has little importance compared to other Andean crops, but it has great potential to be exploited in the industry. For this reason, the objective of this study was to determine the morphological characteristics of the starch, flour and freeze-dried protein of four mashua genotypes grown in the herbarium of the Faculty of Agricultural Sciences in 2018. Using the scanning electron microscope (MEB), the results showed flour granules with spherical, oval shapes with a diameter greater than and less than 6 µm and 5 µm in the yellow genotype (MA); 9.4 µm and 8.6 µm in the white genotype (MB), 8.3 µm and 6.9 in the yellow green genotype (MVA), 9.9 µm and 9.1 µm in the poza rondador genotype (MPR). Starch granules have sizes of 7.1 µm and 6.1 µm (MA), 18 µm and 16.3 µm (MB), 8.3 µm and 7.3 µm (MVA), 7 µm and 6.5 µm (MPR) with spherical, oval and spherical-truncated shapes. While the micrographs of the freeze-dried protein were observed sheets with sizes of 130, 221, 224 and 237 µm (MVA, MA, MB and MPR, respectively). The oval and spherical shape are highlighted in the flour and starch granules and the flattened polygonal shape in the protein, while the largest were the MB and MPR genotypes (starch and flour) and in the case of the MPR protein.

Keywords: Andean, granule shape, size.

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In pre-Columbian times the Andean peoples domesticated about 70 crops in Ecuador, nine species have been studied and one tuber named mashua stands out for its nutritional and medicinal qualities. *Tropaeolum tuberosum* Ruiz & Pavón is the scientific name for this species, which is taxonomically located in the family Tropaeolaceae, genus *Tropaeolum* (Lim, 2016).

The genetic diversity of the tuber is varied but less compared to the oca (*Oxalis tuberosa*) and the olluco (*Ullucus tuberosus*) (Pissard *et al*., 2008), between genotypes are differentiated by their color, shape and flavor (Flores *et al*., 2003). The tuber has an important nutritional composition, with high levels of ascorbic acid (vitamin C), riboflavin (vitamin B2), thiamine (vitamin B1) (National Research Council, 1989), high protein content even higher than potato, oca and olluco (Manrique *et al*., 2013), fiber (Flores *et al*., 2003) and high carotene content (Barrera *et al*., 2004).

The uses of mashua are varied, it stands out in culinary preparations (Barrera *et al*., 2004) and traditional medicine for its bioactive compounds beneficial for health (Campos *et al*., 2006). Andean farmers usually consume the entire plant and especially the tuber (Flores *et al*., 2003) from which they obtain by-products such as flour and starch. From the first processing of mashua as flour, a spicy product is obtained, with a strong-smell and a yellowish appearance, which is classified as unsatisfactory (Grau *et al*., 2003). Ramallo (1999), studied the composition of mashua flour, reporting 8% protein comparable to cornmeal and high energy content 416 kcal 100 g^{-1}, suggesting it as pig food.

On the other hand, starch was noted for its nutritional properties (Grau *et al*., 2003), creating great interest in researchers. Mashua contains 41.35% (BS) starch in the tuber, with 27.44% (BS) amylose (Valcárcel-Yamani *et al*., 2013). Mashua starch has smaller granules compared to tubers such as oca and ulluco, it is composed of 2-3 heterogeneous grains (Surco, 2004). Its shape can become ovoid, spherical and truncated with sizes of 4.39 and 16.29 µm in length and 4.07 and 13.09 µm diameter (Valcárcel-Yamani *et al*., 2013).

According to Valcárcel-Yamani *et al*. (2013) the starch of Andean tubers such as oca, ulluco and mashua are considered of importance in the food industry for the production of products of easy cooking, high viscosity and low stability to refrigeration, but unlike oca and ulluco mashua stands out for its high digestibility when possessing small granules.

The shape and size of mashua starch granules has been discussed by several researchers; however, recent research on the morphology of mashua flour and protein is not yet known, the objective of this research was to characterize morphometrically the starch, flour and protein of four vegetable samples of mashua and look for its differences and similarities with other similar crops.

Plant material

The tubers of four mashua genotypes (*T. tuberosum*) were collected from the germplasm bank of the Faculty of Agricultural Sciences of the Technical University of Ambato. Genotypes were cultivated and harvested in 2018; from four mashua accessions from different geographical origins of Ecuador: the yellow genotype (MA) of the province of Tungurahua, yellow green (MVA) and poza rondador (MPR) in the province of Chimborazo and white (MB) of Cotopaxi province, these correspond to vulgar names acquired by local sources of peasants (Valle-Parra *et al*., 2018).

The tubers were selected for their uniform size and no bruises. Mashua starch extraction. Starch extraction was carried out four days after harvest, using the methods described by Torruco-Uco and Betancur-Ancona (2007), with slight modifications.

The tubers were washed and brushed to remove all impurities (dust, leaves, roots) and then cut into cubes of approximately 3 cm on each side. The cubes were crushed in a blender with distilled water in a ratio of 1:2 (p/v) for 3 min. The resulting suspension was filtered with a muslin cloth to remove the fiber and the filtrate was left to settle at 4 ^{o}C for 4 hours in a precipitation vessel. The starch was separated from the supernatant by centrifugation at 3 000 rpm for 5 min (Melian, 2010) in a Sorvall ST 8 (X-150 Swinging Bucket Rotor) centrifuge, the supernatant was discarded.

The starch obtained was re-suspended in water and left in sedimentation, then it was filtered as described above. This procedure was repeated 3-4 times with distilled water and one last time it was filtered through a cotton layer until obtaining a white starch and a floating translucent. The clean starch was dried on a stove (Modell 100-800) for 24 hours at 37 ^{o}C, ground into a mortar and sieved until obtaining a homogeneous powder. The starch was stored in plastic bottles with a hermetic lid closure and stored at -20 ^{o}C.

Scanning electron microscopy. The structural morphology of starch, flour and protein of mashua was studied using a scanning electron microscope (MEB) (model Tescan Vega3) with an increase of up to 100.000X, the images were made working at 20.0 keV in high vacuum. The samples were prepared and fixed on double-sided pieces of carbon tape and coated with gold using an SPI-Module cathodic spray coating device.

For data tabulation, random measurements of the major or longitudinal diameter (DM), minor or transverse diameter (Dm) and length (L) of starch granule, flour and protein sheets micrographs were performed. Statistical analysis. For statistical analysis, the InfoStat version 2008 program was used, using descriptive statistics, with Tukey test to determine significant differences between averages. Figure 1 details the morphology of starch granules and freeze-dried samples of flour and protein from four mashua genotypes.

In the first column there are micrographs of flour highlighting the oval and spherical shape in the MB, MA and MPR genotypes while in the MVA genotype predominates the oval shape, in addition in all flour samples it can see the presence of particulate matter and the presence of sheets that could correspond to protein. All flour samples have smooth appearance surfaces such as potato starch granules (*Solanum tuberosum*) (Zhou *et al*., 2013), with some cracks similar to maca (Zhang *et al*., 2017) except in MA flour granules where bumps and small grooves similar to corngranules are observed (Singh *et al*., 2003). The size of the MB and MVA granules do not differ significantly otherwise from the MA and MPR genotypes (Table 1).

Table 1. Size and shape of starch granules, flour and protein sheets of four mashua genotypes.

	Flour			Starch			Protein	
Genotypes	Larger diameter (µm)	Minor diameter (µm)	Form	Larger diameter (µm)	Minor diameter (µm)	Form	Longitude (µm)	Form
Yellow (MA)	6.4^{a} ±2.3 (2.8-11.2)	5^{a} ±1.5 (2.5-8)		7.1^{a} ±2.4 (3.2-12.3)	6.1^{a} ±1.6 (3.2-9.1)		205.8^{a} ±60.2 (139.5-257)	Flattened asymmetrical polygon
White (MB)	9.4^{ab} ±2.6 (4-14.1)	8.6^{ab} ±2.3 (3.5-12.6)	Oval	18^{b} ±5.4 (6.7-29)	16.3^{b} ±4.4 (6.7-24)	Oval and spherical	223.7^{a} ±110.9 (125-469.7)	Flattened asymmetrical polygon
Yellow Green (MVA)	8.3^{ab} ±3.1 (3.6-12)	6.9^{ab} ±2.5 (2.7-9.3)	Oval	8.3^{a} ±2.4 (3.8-11.5)	7.3^{a} ±2 (3.8-11.1)	Oval and spherical	129.7^{a} ±35.2 (89-151)	Flattened asymmetrical polygon
Poza rondador (MPR)	9.9^{b} ±4.9 (5-21.7)	9.1^{b} ±4.9 (4.1-21.7)	Oval and spherical	7^{a} ±2.2 (3.6-10.2)	6.5^{a} ±2.2 (3.6-9.5)	Oval and spherical	237^{a}±52.4 (200-297)	Flattened asymmetrical polygon

$^{a-b=}$ different superscripts in the same row indicate significant differences (p> 0.05). Values in brackets indicate the maximum and minimum values observed.

MA flour granules have average diameters of 6.4 µm (DM) and 5 µm (Dm), the MB genotype reports 9.4 µm (DM) and 8.6 µm (Dm), the MVA genotype has sizes of 8.3 µm (DM) and 6.9 µm (Dm) and MPR flour granules report 9.9 µm (DM) and 9.1 µm (Dm). The micrographs of the starch granules are shown in the second column in Figure 1. The starch granules of the four genotypes have a morphology where oval and spherical structures predominate and with less participation the spherical-truncated forms.

These similarities are exposed in the four genotypes, results consistent with other studies, such as yellow mashua granule micrographs reported by Valcárcel-Yamani *et al*. (2013) and other tubers such as makal (*Xanthosoma yucatanensis*) (Torruco-Uco and Betancur-Ancona, 2007) and arrowroot (*Maranta arundinacea*) (Peroni *et al*., 2006).

The size of starch granules is similar between MA, MVA and MPR genotypes, while the MB genotype differs by having larger granules (Table 1). The larger diameters of the granules have averages from 18 µm for the MB genotype, 7.1 µm in MA, 8.3 µm in MVA and 7 µm in MPR and diameters less than 6.1, 16.3, 7.3 and 6.5 µm respectively.

These values are similar to the size of granules reported in yellow mashua genotypes with 4.39 and 16.29 µm (DM) and 4.07 and 13.09 µm (Dm) (Valcárcel-Yamani *et al*., 2013). Also, other similar values have been reported such as maca (*Lepidium meyenii Walpers*) (9 µm), cassava (*Manihot sculpted*) (12.9 µm) and makal (12.4 µm) (Charles *et al*., 2005; Hernández-Medina *et al*., 2008; Torruco-Uco and Betancur-Ancona, 2007; Zhang *et al*., 2017).

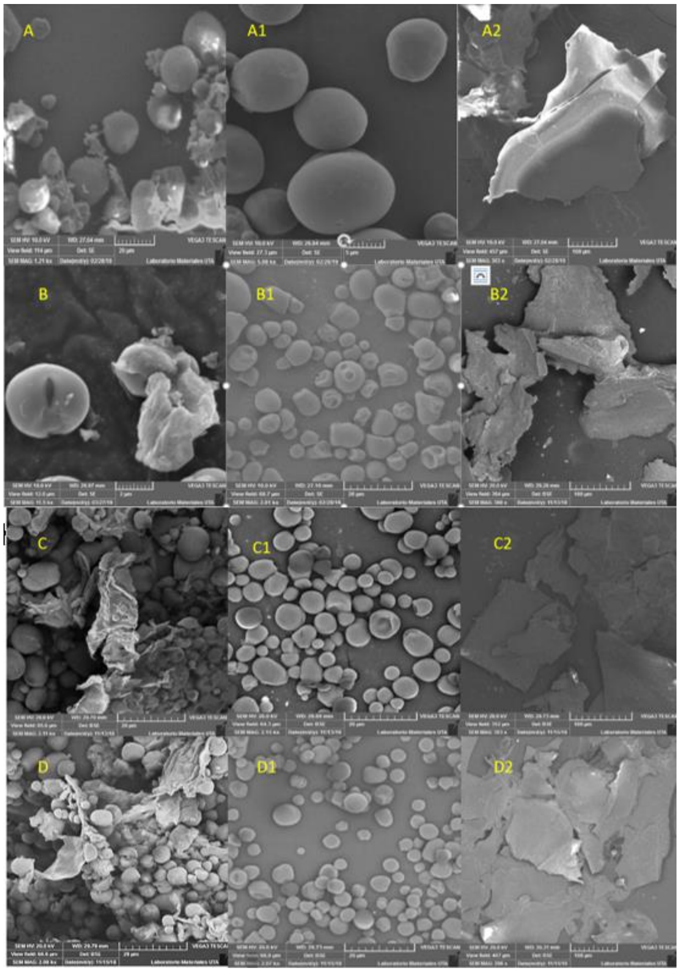

Figure 1. Electronic micrographs (SEM) from left to right: flour, starch and mashua protein genotype MB (A-A2); MA (B-B2); MVA (C-C2); MPR (D-D2) with 100000 X amplification.

Differences in granule size and shape can be attributed to several factors, one of them agriculture, researchers expose that Andean crops over time have undergone changes in domestication and reproduction suggesting an influence on the morphological characteristics and size of the starch granule (King and Gershoff, 1987; Flores *et al*., 2003); however, with modern advances today it is believed that the size of the granule could be related to agricultural practices (Torres *et al*., 2011).

Certain studies attribute the results to the biological origin, biochemistry of chloroplast and plant physiology (Singh *et al*., 2003). While, other authors argue that the morphology and size of starch granules depend on the genetics of the plant, in addition to the membranous structures and physical characteristics of the plastids (Lindeboom *et al*., 2004).

The last column reports protein micrographs of the four mashua genotypes (Figure 1). The shape of the protein is peculiar as they are not spherical or oval but laminar structures with brittle surface and asymmetrical polygonal shape. These sheets have different average lengths ranging from 130, 221, 224 and 237 µm (MVA, MA, MB and MPR, respectively) (Table 1), its shape resembles the beach pea albumin (*Lathyrus maritimus* L.) with plate topography and smooth surface (Chavan *et al*., 2001) and the bean albumin (*Phaseolus vulgaris* L.) Great Northern variety in the shape of rod (Sathe and Salunkhe, 1981). Past research has indicated that the topographical characteristics of proteins could intervene in the physicochemical and functional properties of starch (Chavan *et al*., 2001), but being at low levels would have no negative effects according as exposed by Hoover (2001).

Conclusions

The study of starch morphology showed oval and spherical shapes similar to flour granules, but different from the freeze-dried proteins with laminar structures with asymmetric polygonal shape, regarding the size of the starch and flour granules, it was observed that the MB and MPR genotypes were the ones that stood out in the largest size respectively, while the protein sheets had significant differences being the largest MPR genotype.

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