



## **Compositional characteristics of amaranth species used as vegetables**

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#### **Abstract**

There are few studies on the nutritional quality of young plants of the different species of the genus Amaranthus. The objective was to evaluate the nutritional quality of plants of the species Amaranthus hypochondriacus L., A. cruentus L., and A. hybridus L. grown in the municipalities of Zapotitlán de Méndez and Tochimilco, in the state of Puebla and the municipality of Temoac, Morelos, Mexico. The evaluation included forty-three genotypes classified by species and use in 11 accessions of A. hypochondriacus for grain, 12 of A. hypochondriacus for vegetables, 8 of A. cruentus for grain, and 12 of A. hybridus for vegetables. The trial was established in 2019 in Zapotitlán de Méndez, Puebla, Mexico. The plants were cut 40 days after sowing, then dried, ground and stored to perform a proximate analysis to the samples. It was found that there were no differences ( $p \leq 0.05$ ) between accessions with use as grain and as vegetables in most of the variables measured, except for neutral detergent fiber and dry stem weight. Between species, there were only differences (p≤ 0.05) in acid detergent fiber, neutral detergent fiber, and ash. Between accessions, there were differences ( $p \le 0.05$ ) in all variables, except for dry leaf weight, dry stem weight, and total dry weight. The outstanding accessions to be used as vegetables due to their high content of crude protein and low content of acid detergent fiber and dietary fiber were AV17, AV28, AV29, AV31 and Benito.

#### **Palabras clave:**

Amaranthus cruentus, Amaranthus hybridus, Amaranthus hypochondriacus, Mexico.



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## **Introduction**

Amaranth is used for grain production; it is also used as a vegetable, forage, colorant, and ornamental plant (Brenner et al., 2000; Morales et al., 2014). The species that have been used as grain in Mexico are A. hypochondriacus L. and A. cruentus L. (Espitia-Rangel et al., 2010). Young plants of different species are used as vegetables, among which the following stand out: A. hybridus L., A. retroflexus L., A. palmeri S. Wats., A. powellii S. Watz., A. dubius, and A. spinosus L. (Mapes et al., 2012a).

The advantages of consuming the amaranth plant as a vegetable are the following: it has high protein content as levels from 13.6 (Seguin et al., 2013) to 33.5% (Morales et al., 2014) have been reported; the leaves contain high percentages of calcium, iron, phosphorus and magnesium, ascorbic acid, vitamin A, and fiber (Brenner et al., 2000; Das, 2016). Nutritional quality varies depending on the species (Mapes et al., 1996; Mapes et al., 1997; Brenner et al., 2000), genotype (Shukla et al., 2006), plant age (Pospišil, et al., 2009), plant part (García-Pereyra, 2009), and agronomic management (Abbasi et al., 2012).

The Sierra Norte de Puebla is one of the regions in Mexico where there is genetic diversity, productive tradition, and culture of consumption of amaranth as a vegetable (Mapes et al., 2012b; Mapes et al., 2013). In this area, among the species used as vegetables are A. hypochondriacus and A. hybridus (Mapes et al., 2013).

On the other hand, the amaranth grain production area in Mexico is located in the center of the country, mainly in the state of Puebla, and the predominant species is A. hypochondriacus. Amaranth is also produced for grain in the state of Morelos with the species A. cruentus.

Despite the fact that in Mexico, there is a wide genetic and cultural diversity of consumption of amaranth as a vegetable among the population, there is no information regarding the nutritional quality of the plant of the different species that exist in the country.

A study focused on specifying the nutritional quality of the different species used for vegetable production could direct research for the purpose of selecting species and accessions with higher nutrient content and palatability for use in a breeding program and increase the benefits of growing and consuming amaranth for this purpose among the population. Therefore, the objective was to evaluate the nutritional quality of plants of the species Amaranthus hypochondriacus L., A. cruentus L., and A. hybridus L. grown in the municipalities of Zapotitlán de Méndez and Tochimilco, Puebla and Temoac, Morelos, Mexico.

# **Materials and methods**

A total of 43 amaranth genotypes were evaluated, of which 24 were populations used as vegetables, collected in the municipality of Zapotitlán de Méndez, Puebla. This municipality is located between parallels 19° 59' and 20°02' north latitude, meridians 97° 39' and 97° 44' west longitude and has an average altitude of 659 m. In addition, 10 improved varieties and nine populations used for commercial grain production were evaluated (Table 1). Grain populations were collected in the municipalities of Tochimilco, Puebla and Temoac, Morelos, Mexico.

**Table 1. Code, species, type of material, and use of amaranth genotypes evaluated in the SS/2019 cycle in** Zapotitlán de Méndez, Puebla.





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The municipality of Tochimilco is located between parallels 18° 50' and 19° 02' north latitude, meridians 97° 18' and 97° 27' west longitude and has an average altitude of 2 060 m and an annual rainfall of 900 mm. The municipality of Temoac is geographically located at 18° 46' 20" north latitude and 98° 46' 39" west longitude, at an average altitude of 1 580 m and with an annual rainfall of 857 mm. The code, species, type of material, and use of the amaranth genotypes evaluated are presented in Table 1.

The preparation of the land consisted of eliminating the weeds present, removing the earth with a hoe, and forming furrows 25 cm wide. The experimental plot consisted of two rows 25 cm wide by 5 m long. The sowing was carried out on September 22, 2019; 2 g of seed per plot was deposited at a steady flow and covered with a layer of one centimeter of soil. Chemical fertilization was not applied because the local management given to the crop was followed. The experimental design used was randomized blocks with two replications.

The sowing was established in the locality of Zapotitlán, in the municipality of Zapotitlán de Méndez, Puebla, Mexico, which is geographically located at 20° 0' 30.377" north latitude and 97° 42' 52.056" west longitude. The municipality has a semi-warm sub-humid climate with rainfall all year round, with temperatures of 18 to 22 °C, and an average annual rainfall ranging from 2 000 to 2 500 mm (INEGI, 2015).

The plots were harvested 40 days after sowing. The seedlings were cut 2 cm from the surface, stems and leaves were separated, placed in a paper bag, and dried in a forced-air oven (Thermo Scientific) at 55 °C for 48 h. All samples were ground in a Foss Tecator® cyclone mill with 1 mm mesh and stored in bags (Ziploc®) for later analysis. The analyses were carried out in the laboratory of the College of Postgraduates, Puebla Campus, during 2020.

## **Study variables**

In each plot, all the seedlings were harvested and the leaves and stems were separated and dried in a forced-air oven (Thermo Scientific) at 55 °C for 48 h, and the dry leaf eight (DLEW) and the dry stem weight (DSTEW) were determined in grams. The total dry weight (TDW) is the sum of the DSTEW plus DLEW, expressed in grams. Acid detergent fiber (ADF), neutral detergent fiber (NDF), and lignin (LIG) were established using a sequential determination following the Ankom Technology (2006) protocols for fiber analysis.

NDF determination was performed in duplicate with 0.5 ±0.0015 g sample on an Ankom 200/220 fiber analyzer (Ankom, Macedon, NY, USA). Sequentially, the ADF determination was made in duplicate and finally, the lignin determination was carried out with a concentration of sulfuric acid  $(H<sub>2</sub>SO<sub>4</sub>)$  at 72%. The determination of crude protein (CP) was carried out by the Micro Kieldahl method and was performed in triplicate. Zero point two grams of sample and 0.8 g of selenium were weighed in a test tube, and 3 ml of sulfuric acid was added.

They were placed in the digester (Kjeldahl DIK-20 digester, Prendo) at 350 °C and after two and a half hours, the sample was verified until it presented a transparent light green color. Ten milliliters of distilled water and 10 ml of NaOH were added to each tube. They were placed in the distiller, receiving the distillate in a 5 ml beaker containing 5 ml of  $H_3BO_4$  with five drops of tashiro. It was distilled until 20 ml was obtained and each distillate was poured into an Erlenmeyer flask to be titrated with 0.1 N HCL.



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Total dietary fiber (TDF) was quantified using the Dietary Fiber Assay Kit (TDF100A, Sigma-Aldrich, St. Louis, Missouri, USA), which is based on the enzymatic-gravimetric methodology described by the AOAC (AOAC, 1997), with some modifications. One gram of sample was used and it was done in duplicate.

Fifty milliliters of pH 6 phosphate buffer and 0.1 ml of α-amylase were added to each sample and they were placed in a water bath at 95 °C for 15 min. It was cooled to room temperature, adjusting to a pH of 7.5 with 10 ml of NaOH at 0.275 N. It was added 0.1 ml of a 50 mg ml<sup>-1</sup> protease solution in a phosphate buffer and incubated in a water bath at 60 °C for 30 min. It was cooled to room temperature and adjusted to pH 4-4.6 by adding 10 ml of HCl at 0.325 N. Finally, 0.1 ml of amyloglucosidase was added and it was left in a water bath for 30 min at 60 °C.

The solutions of each sample were vacuum filtered on filter paper and four volumes of 95% ethanol were added to each supernatant. Each of the solutions was vacuum filtered in gooch crucibles (previously placed at constant weight), to which 0.5 g of celite was previously added; the celite was moistened with 78% ethanol and the residue was washed with 30 ml of 78% ethanol, 20 ml of 95% ethanol and 20 ml of acetone. The crucibles were dried in an oven (Shell Lab, Sheldon, Cornelius, OR, USA) at 100 °C and the weights were recorded.

The following formulas were used to quantify dietary fiber: insoluble fiber= weight of the filter paper with fiber-dry weight of filter paper. The (%) insoluble fiber= (insoluble fiber/grams of sample used)  $x$  100. Soluble fiber= dry weight of the crucible + celite + dry sample of the crucible with celite.  $%$ soluble fiber= (soluble fiber/grams of sample used) x 100. Total dietary fiber= % insoluble fiber + (%) soluble fiber.

The ash (ASH) content in the samples was measured in accordance with the Mexican Standard NMX-F-066-S-1978.

Soluble carbohydrates (CAB) were determined using the Clegg-Anthrone method (Thimmaiah, 1999). Zero point five grams per sample were weighed and placed in an Erlenmeyer flask, 10 ml of distilled water was added to it and it was stirred. Subsequently, 13 ml of perchloric acid solution was added to the flask and it was stirred in an orbital shaker (Orbit 1900, Labnet International Inc., USA) for 20 min. It was made up to 50 ml with distilled water and filtered. Next, 10 ml of the extract was diluted to 100 ml with distilled water.

One milliliter of the diluted filtrate was transferred to the test tube and 5 ml of freshly prepared anthrone reagent was immediately added. The tubes were covered and placed in a water bath for 20 min at 100  $\degree$ C. The tubes were cooled to room temperature and read in the spectrophotometer at 630 nm.

## **Statistical analysis**

The data obtained were subjected to an analysis of variance and a mean comparison test (Tukey). The statistical model used was a nested three-state design described by Montgomery (2013):  $y_{ijkl}$ =  $\mu$  +  $\tau_i$  +  $\beta$  <sub>j(i)</sub> +  $\eta_{k(ij)}$  +  $\varepsilon_{(ijk)l}$ . Where:  $y_{ijkl}$  is the observed value of the study characteristic;  $\mu$  is the overall mean of the experiment; τ<sub>i</sub> is the effect of the i-th use;  $β_{j(i)}$  is the effect of the j-th species within the i-th use;  $\eta_{k(ij)}$  is the effect of the k-th genotype within the j-th species and the i-th use; and  $\varepsilon_{\text{(ik)}}$  is the error term. The analyses used the SAS program, version 9.3 (SAS Inst., 2011).

# **Results and discussion**

Table 2 shows the mean squares of the characteristics evaluated in amaranth populations. In the variation factor of use, there were only highly significant statistical differences (p≤ 0.01) in DSTEW and ADF. In the variation factor of species nested in use [S (U)], there were highly significant differences ( $p \le 0.01$ ) in NDF and ADF and significant differences ( $p \le 0.05$ ) in ash. In the factor of treatment nested in use and species [T (U S)], only in the response variables of DLEW, DSTEW, and TODW, there were no significant differences between the accessions evaluated; in the rest, there were highly significant differences ( $p \leq 0.01$ ).



Table 2. Mean squares of the use, species, and treatment of the variables evaluated in amaranth populations and varieties. Zapotitlán de Méndez, Puebla, 2019.



DF= degrees of freedom; S (U)= species (use); T (U S)= treatment (use species); DLEW= dry leaf weight; DSTEW= dry stem weight; TODW= total dry weight; NDF= neutral detergent fiber; ADF= acid detergent fiber; TDF= total dietary fiber; CAB= carbohydrates; CP= crude protein; CV= coefficient of variation. \*, \*\* = statistically significant at the 0.05 and 0.01 probability levels, respectively; ns= not significant.

## **Means by use**

The populations or varieties of amaranth used to produce grain were not significantly different from those whose main use is to produce vegetables (Table 2), in terms of content of CP, NDF, LIG and TDF, ASH, DLEW, and TODW. This information contributes to consider that all the amaranth species studied have similar plant quality. However, it was found that species used for grain, compared to amaranths used for vegetables, had higher concentrations of ADF and DSTEW.

In the analysis of means by use, it was found that, in the DSTEW variable, amaranths for grain (45.7 g) had a higher weight ( $p \le 0.05$ ) than species with vegetable use (34.2 g). According to Mapes et al. (1997), species used as vegetables have higher leaf production due to the selection made by man. In the ADF variable, amaranths for grain have a higher value (26%) ( $p \le 0.05$ ) than vegetable amaranths (23.4%). This characteristic gives an indication of better palatability of vegetable species.

This is consistent with what was reported by Mapes et al. (1996); Mapes et al. (1997); Brenner et al.(2000), who mention that amaranths used as vegetables have better characteristics for consumption as tender plants, such as better palatability. Nonetheless, Stodahl et al. (1999), when evaluating accessions for grain and vegetables, found that accessions differed in quality; for example, higher crude protein content and lower NDF and ADF, but the differences were not related to the type of amaranth (grain or vegetable) but were independent of their main use for which they are intended, since there were outstanding populations in quality in both types of use.

## **Means by species**

When comparing seedling quality between the different species (Table 3), it was found that the three species evaluated had the same contents of DLEW, DSTEW, TODW, CP, Lignin, TDF, and CAB. Nonetheless, there were significant differences in NDF, ADF, and Ash contents.









Spe= species; cruen= *A. cruentus*; hypo= *A. hypochondriacus*; hybri= *A. hybridus*; G= grain; V= vegetable; CP= crude protein; NDF= neutral detergent fiber; ADF= acid detergent fiber; LIG= lignin; TDF= total dietary fiber, ASH= ashes; CAB= carbohydrates; DLEW= dry leaf weight; DSTEW= dry stem weight. Means with the same letter in the same column are statistically equal ( $p \le 0.05$ ). LSD= least significant difference.

In NDF, A. hypochondriacus species for grain and vegetables had the highest values, with 42 and 43.3%, respectively, which exceeded ( $p \le 0.05$ ) those of A. cruentus (40.1%) and A. hybridus (38.9%). The levels of NDF found are similar to those reported by other researchers. Seguin et al. (2013) observed concentrations between 37.2 and 40.15% in A. hypochondriacus. Pospišil et al. (2009), who studied three different stages of development of A. hypochondriacus, found values in the range of 42.3 to 47.8% NDF. In A. hypochondriacus and A. cruentus; Stordahl et al. (1999) reported 35%. Sleugh et al. (2001) found 31 to 43%; García-Pereyra et al. (2009) calculated stem contents from 66.4 to 73.1% and leaf contents from 38.2 to 47.4%.

In ADF, A. hypochondriacus for grain presented the highest value with  $27.9\%$  ( $p \le 0.05$ ). In second place were A. hypochondriacus for vegetables and A. cruentus with 24.9 and 24%, respectively, while A. hybridus had a content of 22%. The lower ADF values of A. hybridus are consistent with those reported by Mapes et al. (1997) in species used for vegetables and are an indication of better palatability.

Other researchers have reported levels of ADF similar to those found in this study. Seguin et al. (2013) reported 24.7-29.1% in A. hypochondriacus. Abbasi (2012) found between 20.7-21.5% in this same variable; Pospišil et al. (2009) reported 27.4 to 36.6% in three different stages of plant development. In works where they studied A. hypochondriacus and A. hybridus; Sleugh et al. (2001) observed 17.4% at 42 days after emergence and Stordahl et al. (1999) found 26% in the eighth week. For their part, García-Pereyra et al. (2009), in A. hypochondriacus and A. cruentus, found 66.4-73.1% in the stem and 38.2-47.4% in leaves.

Some authors report that human selection has led to more palatable plants in the populations used to produce vegetables compared to those used for grain production since the latter focus their growth on seed production (Mapes et al., 1996; Mapes et al., 1997; Brenner et al., 2000).

In ash, the species with the highest content ( $p \le 0.05$ ) were A. hybridus and A. hypochondriacus for grain with 20.7 and 20.4%, respectively. In second place were A. cruentus for grain and A. hypochondriacus for vegetables with 20.1 and 19.9%, respectively. These values are similar to what other researchers report. Morales et al. (2014) found 16.1 to 21.6% in A. cruentus, 21.2% in A. hypochondriacus, and 23 to 28.3% in A. hybridus. Likewise, García-Pereyra et al. (2009), in A. hypochondriacus and A. cruentus, obtained 16.3-18.9% in the stem and 23 to 28.3% in leaves.

## **Means by accessions**

It was found that the content of CP was between 11.1 and 17.8% (Table 4), with an overall mean of 14.3%. A group formed by 14 populations and improved varieties had the highest protein levels  $(p# 0.5)$  with values between 14.9 and 17.8%. This group included representatives of the three species and the two uses evaluated. Of the species A. hypochondriacus for grain were CP30, CP2, Gabriela, Nutrisol, Areli and PQ2.







Spe= species; V= vegetable; G= grain; hypo= *A. hypochondriacus*; crue= *A. cruentus*; hybr= *A. hybridus*; CP= crude protein; NDF= neutral detergent fiber; ADF= acid detergent fiber; TDF= total dietary fiber; ASH= ashes; CAB= carbohydrates; DLEW= dry leaf weight; DSTEW= dry stem weight.  $\dot{\bar{}}$  = means belonging to the statistically superior group ( $p \le 0.05$ ). LSD= least significant difference.

In A. hypochondriacus for vegetables, it was AV28. In A. cruentus, they were CP39, Amaranteca, and Benito. In A. hybridus, they were AV17, AV29, AV18, AV31, and AV9. This result reinforces that the consumption of amaranth as a vegetable is an important source of healthy plant-based protein for human consumption. In general, the observed mean is at the level reported by other researchers. Morales et al. (2014) reported values of 20.9 to 33% in A. cruentus, 21.6% in A. hypochondriacus, and 22.1 to 33.5% in A. hybridus.

Various values have been reported in A. hypochondriacus; for example, Seguin et al. (2013) determined 13.6 and 15.2%; Pospišil et al. (2009) 15.2 to 21.6%, and Abbasi et al. (2012) from 24.3 to 26.5%. This wide variation in protein content is due to the environment, species, management, and age of the plant in which the determination was made (Stodahl et al., 1999; Sleugh et al., 2001; Pospišil et al., 2009).

The NDF values found were between 32.8 and 49%, with a mean of 41.2%. The lowest NDF values were found in the following species and accessions. In A. hypochondriacus (vegetable): AV28, AV12, and AV21. In A. hybridus: AV20, AV17, AV30, AV19, AV29, AV13 and AV31. In A. cruentus: CP39, Amaranteca, CP15, CP34 and CP40. In contrast, in A. hypochondriacus for grain, there was no accession with low NDF level. Several researchers consider that the low levels of NDF present in amaranth make it an option for human consumption and as forage for ruminants (Stordahl et al., 1999; García-Pereyra et al., 2009; Pospišil et al., 2009).



The ADF values were between 14.3 and 34.6% and the mean was 24.7%. The group with the lowest amount of ADF was formed by populations AV28, AV12, and AV21 of A. hypochondriacus for vegetable, AV30, AV20, AV17, AV29, AV9, AV13 and AV31 of A. hybridus, and CP15 and Benito of A. cruentus; there were no outstanding accessions of A. hypochondriacus for grain.

The lignin values ranged from 1.6 to 8%, with a mean of 4.2%. For this variable, the group of accessions with the lowest content (p≤ 0.5) was made up of populations or varieties of different species and use: the genotypes AV25, AV12, AV14, AV3, and AV24 in A. hypochondriacus for vegetable, AV29, AV13, and AV31 in A. hybridus, CP2, CP43, and Revancha in A. hypochondriacus for grain, and there was no outstanding representatives in A. cruentus.

The values obtained were similar to those reported by Seguin et al. (2013) in A. hypochondriacus, who reported values between 2.5 and 3.1%. Sleugh et al. (2001) in A. hypochondriacus and A. hybridus, who determined values of 1.7 to 2.9% at 42 days after emergence; on the other hand, Abbasi (2012) found levels of 2.5 to 2.68% in A. hypochondriacus. The variation in Lignin may be due to genotype, production conditions, plant age and selection by growers.

TDF values ranged from 62.16 to 78.87% in the 43 genotypes evaluated. The genotypes with the highest TDF average (p≤ 0.5) were between 74.7 and 78.8%. This group was made up of AV6 and AV25 of A. hypochondriacus for vegetables, AV8, AV9 and AV13 of A. hybridus, CP39 of A. cruentus and CP30, Areli and Gabriel of A. hypochondriacus for grain. Based on the TDF results, it was found that the samples have highly fermentable fiber because they contain high amounts of soluble and insoluble fibers.

A good dietary fiber content in foods is important because a consumption of 18-38 g per day is recommended for optimal health status in adults; however, in Mexico, such requirements are not met (Vilcanqui-Pérez and Vílchez-Perales, 2017). The ash contents ranged from 16 to 21% and they were similar to those found in various studies; Wesche-Ebeling et al. (1995) reported levels of 16.9 to 22.1% in A. retroflexus, A. palmeri and A. blitoides. Onyango et al. (2008) found 19.2% on average in various amaranth species.

In CAB, the means of accessions ranged from 1.5 to 8.4%. The outstanding group was made up of 20 genotypes of all the species and use evaluated; these had an average between 5.7 and 8.4%. The values found in this study were similar to the 4.1% reported by Nehal et al. (2016) in A. lividus; to the 3.4% by Asaolu et al. (2012) in A. hybridus and to the 7% by Mensah et al. (2008) in A. cruentus. The variation observed in CAB is explained by the fact that the levels of these compounds are influenced by the sowing, growth, handling, and fertilization conditions of the plant (Marshall, 1985).

The statistical differences (p≤ 0.05) between accessions in all the variables measured and the existence of genotypes that are statistically superior in contents of CP, NDF, ADF, LIG, TDF, ASH and CAB, show the diversity in quality of the seedlings that exist in the different amaranth species. This diversity is consistent with previous studies as morphological diversity has been reported within amaranth species for vegetables (Mapes et al., 2012a; Mapes et al., 2013).

The selection of amaranth materials with seedlings that have a high level of protein and low contents of ADF and NDF can result in a production of vegetables of high quality and palatability; in this case, one could use AV28 of A. hypochondriacus vegetable, AV17, AV29, AV31 of A. hybridus and the Benito variety of A. cruentus.

# **Conclusions**

Among amaranth accessions classified by type of use, no differences were observed between materials in most of the variables evaluated; nevertheless, accessions with grain use had higher concentration of acid detergent fiber and dry stem weight compared to accessions used for vegetables. Among accessions classified by species and use, it was observed that they present similar contents in most of the variables evaluated. Nonetheless, the species A. hypochondriacus of grain and vegetable type presented high values of neutral detergent fiber, while A. hypochondriacus for grain had the highest values of acid detergent fiber.



When the collections or varieties were analyzed, without considering species or use, a great variation was found in most of the variables evaluated, except in dry leaf weight, total dry weight, and dry stem weight, which evidences the existence of a wide genetic diversity within the different species studied. The outstanding materials to be used as vegetables due to their high contents of crude protein and low contents of acid detergent fiber and dietary fiber were AV17, AV28, AV29, AV31 and Benito.

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