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Full Length Article

Comparative Account of Phenolics, Antioxidant Capacity, α-Tocopherol and Anti-nutritional Factors of Amaranth (*Amaranthus hypochondriacus*) Grown in the Greenhouse and Open Field

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Abstract

The Amaranth is a plant considered as a third millennium crop due to its nutritional properties and its agronomic versatility. Amaranth (*Amaranthus hypochondriacus* L.) can be cultivated in greenhouses or the open field. Greenhouse cultivation provides better yields but may affect chemical composition. In this study we compared antioxidant capacity, content of total phenolic compounds, condensed tannins, anthocyanins, α-tocopherol and anti-nutritional factors (phytic acid and trypsin inhibition) in amaranth plants grown in the greenhouse and in the open field. An area of 100 m² was used for each cultivation system. Cultures were performed by direct sowing in furrows, with a system of drip irrigation and no fertilizers were used. Student's *t* test was used for comparison of variables in the two systems. Greenhouse-grown amaranth showed the highest values for total anthocyanins in leaf and α-tocopherol (leaf and seed), while in the open-field system, higher values of trypsin inhibition, antioxidant capacity, total phenolic compounds and condensed tannins were found in leaves and seed.

Keywords: Amaranth; Greenhouse; Anti-nutritional factors; Phenolic compounds; Antioxidant capacity; α-tocopherol

Introduction

Mesoamerica has been the cradle of genetic diversity and source of some of the most important food crops for humanity. Corn, beans, and amaranth are just a sample of the food wealth that this region has brought to the world. Amaranth was domesticated in the Americas over 4,000 years ago by pre-Hispanic cultures and has been rediscovered as promising food crop due mainly to its resistance to heat, drought, disease and pests, and the high nutritional value of both seeds and leaves (Sogbohossou and Achigan-Dako, 2014). The Amaranthaceae family consists of 60 genera and about 800 species. Sixty of these species are cosmopolitan and grow particularly in areas of human activities where they are regarded as weeds (Corke *et al.*, 2016). Of these 60 species, only three are considered good seed producers: *Amaranthus hypochondriacus* (the main variety grown in Mexico), *A. cruentus* and *A. caudatus* (Bressani, 2003).

Amaranth is a dicotyledonous plant, an annual species of fast-growing herbaceous or shrub plants of different colors, and is reproduced by self-pollination, mainly by the wind. It is resistant to drought and has the ability to grow in open-field conditions and to withstand different climates (Morales *et al.*, 2009; Vargas-Ortiz *et al.*, 2015). The amaranth plant can be utilized in a wide variety of forms such as seed, vegetable, fodder, or for industrial uses (Breene, 1991; Khandaker *et al.*, 2010; Ramos-Díaz *et al.*, 2013). Amaranth leaf is known to contain high levels of calcium, iron, phosphorus and magnesium, as well as ascorbic acid, vitamin A and fiber, beating spinach values. Amaranth seed has interesting nutritional characteristics as well. One of the most important is the content and quality of protein (Fidantsi and Doxastakis, 2001; Venskutonis and Kraujalis, 2013; Orona-Tamayo and Paredes-López, 2017). Interest in the way amaranth is cultivated and consumed has

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grown in the past two decades because of favorable reports of its nutritional value and health benefits. One line of research on amaranth is the study of how nutritional composition may be related to the form of cultivation (Pospisil *et al.*, 2006; Sarmadi *et al.*, 2016).

Food production methods rely heavily on the infrastructure available for obtaining the highest performance at the lowest possible cost (Silverstone, 1997). In this sense, greenhouses can intensify agricultural production by providing appropriate conditions for accelerating the development of crops and producing more biomass per unit of cultivated area, compared to what can be obtained from crops in the open field (Serrano-Arellano *et al.*, 2015). Furthermore, as structures to protect crops, greenhouses allow them to develop with little risk to production, unlike crops in the open field, which are exposed to environmental variations and depend on the randomness of natural factors. The use of greenhouses allows for more efficient watering and supplies, greater control of pests, weeds and diseases (Luedtke *et al.*, 2003; Fuller and Zahnd, 2012; Stoknes *et al.*, 2016).

However, it is also necessary to evaluate variations in the nutrient composition and the production of secondary metabolites (i.e., phenolic compounds) in these plants, as they are of particular interest to human health. It is known that the cultivation of greenhouse amaranth produces higher yields than open-field cultivation. But this comes at the cost of its nutritional quality (Chávez-Servín *et al.*, 2017). In the existing scientific literature, there are no studies into the difference in content of secondary metabolites in amaranth crops produced in greenhouses and amaranth crops produced in open field. The aim of this study is to compare the content of phenolic compounds, antioxidant capacity, α-tocopherol and anti-nutritional factors in leaves and seeds of amaranth (*A. hypochondriacus*) grown in the greenhouse and the open field.

Materials and Methods

Experimental Details and Treatments

Location, type of greenhouse and cropping systems: The study was carried out at the Amazcala Campus of the Autonomous University of Querétaro, located in the municipality of El Marques, state of Queretaro, Mexico CP 76130. Amazcala is situated at 20° 42' 20 " north and 100° 15' 37" west, 1,921 m above sea level. An area of 100 m^2 was used for the open-field cultivar and another area equal for greenhouse cultivation. For greenhouse and open field cultivation, Eurodrip irrigation lines (diameter: 8000) were used with high turbulent flow emitters and a coefficient of variation <2%. The distance between drip points was 20 cm, with a flow rate of 0.4 GPH (USA, Eurodrip, Guanajuato, Mexico). Automatic irrigation systems were used in both crops, with 5 irrigations every 8 min from the plant's emergence until day 90, and 2 irrigations every 8 min between 90 and 115 days after sowing. The production cycle was spring-summer in the months of March to July (2016) for both cropping systems. The type of soil used was the same for both cropping systems. Seeds of the species amaranth (*Amaranthus hypochondriacus* L. var. Revancha) were used. For each cultivation system - open field and greenhouse - direct sowing was carried out. In both cultivation systems, the soil was loosened using a backhoe. The digging of furrows, leveling of the soil and sowing were done manually. The distance between rows was 50 cm with a depth of 11 cm. A culture density of 15 plants per 2 m (150,000 plants/ha) was established. The characteristics of the greenhouse and type of soil were the same as those previously reported (Chávez-Servín *et al.*, 2017). Temperature (°C) and relative humidity (%) in the greenhouse and open field were monitored using a sensor from Spectrum Technologies, INC. (Micro Stations WatchDog 1000 Series).

Amaranth samples: Leaf samples were collected at 48, 58 and 76 days after sowing. The seed harvest was performed 100 days after sowing. The samples were dried (40°C for 2 days) and ground to a particle size ≤0.05 mm for further analysis. Methanol extraction (methanol-water 80:20 v/v) was carried out as previously reported (Chávez-Servín *et al.*, 2017).

Analytical Determinations

Determination of phytic acid: For the analysis of the content of phytic acid in leaf and seed amaranth samples, we used the method of Vaintraub and Lapteva (1988), modified by Gao *et al.* (2007). Briefly, 0.5 g of sample was weighed and 10 mL of HCl (3.5%) were added. The samples were shaken for 1 h and centrifuged at 5,000 rpm for 20 min at 10°C. The supernatant was removed and 1 g of NaCl was added. Subsequently, it was stirred at 350 rpm for 20 min. The resulting solution was placed at -20°C for 20 min and centrifuged at 5,000 rpm for 20 min at 10°C. Supernatant (1 mL) was diluted with deionized water and Wade reagent was added. Subsequently, it was centrifuged at 5,000 rpm for 10 min at 10°C. The supernatant was read at 500 nm and extrapolated with a reference curve of sodium phytate.

Determination of trypsin inhibitory activity: For analysis of the trypsin inhibitory activity in leaf and seed amaranth samples, the Liener (1977) method was used. The dry sample (1 g) was extracted in 50 mL of NaOH solution (0.01 N, pH 9.5). Afterwards, it was macerated with a homogenizer for 2 min and left overnight while stirring at 4°C. Subsequently, the extract was centrifuged at 3,600 rpm for 15 min at 20°C. The supernatant was collected and diluted until it had a trypsin inhibitory activity close to 50%. The inhibitory activity was observed in 0.5 mL of sample with 1.25 mL of benzoyl-DL-arginine-p-nitroanilidahydrochloride, and incubated for 10 min at 37°C. Subsequently, 0.5 mL of trypsin was added, shaken and re-incubated for another 10 min at 37°C and then

0.25 mL of 30% acetic acid was added. The resulting solution was centrifuged at 12,000 rpm for 12 min. The supernatant was read at 385 nm against a blank of distilled water. The results are expressed as mg of pure trypsin inhibited/g dry matter.

Determination of antioxidant capacity by 3-ethylbenzothiazoline-6-sulphonic acid (ABTS): The commercial kit Cayman ABTS antioxidant assay, item No. 7099001, was used and the manufacturer's instructions were followed. The method is based on the ability of antioxidants in a sample to inhibit the oxidation of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) or ABTS by methyoglobin. The antioxidants in the sample cause suppression in the absorbance at 750 nm in proportion to their concentration.

Determination of antioxidant capacity by the ferric reducing/antioxidant power (FRAP) method: The method of Benzie and Strain (1996) was used at 37°C and pH 3.6, measuring the absorbance after 30 min at 593 nm. The method is based on ferric to ferrous ion reduction (Fe^{3+} to Fe^{2+}), which causes the formation of an intense blue ferrous-tripyridyl-s-triazine complex (TPTZ) that can be read at an absorbance of 593 nm. The absorbance is proportional to the combined ferric/antioxidant reducing power of the antioxidants in the amaranth samples. The results were expressed in grams of ascorbic acid (AA) equivalents per 100 g of dry matter (DM).

Determination of Antioxidant Capacity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method: An assay of DPPH was carried out according to the method reported by Brand-Williams *et al*. (1995), with some modifications to incorporate use a microplate reader. Aliquots of 280 μL of 100 µM DPPH/methanol solution were used per well in the same row of a 96-well plate. Subsequently, 20 μL of extracts diluted in different concentrations were added to complete 300 μL in each well. 300 µL aliquots of methanol were used in the first row as a blank. The plates were incubated for 30 min in the dark and the absorbance at 490 nm was measured.

Determination of total phenolic compounds: Samples was measured by the Folin–Ciocalteu method (Singleton and Rossi, 1965). Results were expressed in mg of gallic acid (GA) equivalents/100 g of DM.

Determination of total anthocyanins: The method published by Abdel and Hucl (1999) was used. Fresh 1 g sample was placed in 25 mL of ethanol solution and stirred at 8,000 rpm for 30 min. The pH was adjusted to 1 with 4 N HCl and centrifuged at 5,000 rpm. The supernatant was decanted and centrifuged again for 10 min. An aliquot was taken and the absorbance was read at 535 nm.

Determination of condensed tannins: They were measured by a vanillin hydrochloric acid test (Deshpande and Cheryan, 1985). The results were expressed in mg of catechin equivalents/100 g of DM.

Determination of α-tocopherol: The determination of α-tocopherol was performed by RP-HPLC-DAD method (Gimeno *et al*., 2000) with slight adaptations. After weighing out approximately 0.25 g or 0.50 g of leaf or amaranth seed, respectively, 500 μL of HPLC grade methanol was added and mixed by vortexing for 3 min. An 800 μL of HPLC grade hexane were then added and mixed by vortexing for 3 min. The mixture was centrifuged at 4,000 rpm for 10 min at a temperature of 5°C. The hexane phase was recovered and taken to dryness using a stream of nitrogen. It was then reconstituted with 100 μL of ethanol HPLC grade and vortexed for 3 min. The extracted sample was injected into the HPLC system. We used a Waters 2996 liquid chromatographic system with a Waters 1525 binary HPLC pump and a Waters 717 plus autosampler injector (Milford, MA, USA). We used a pre-column guard Resolve C18 cartridge (90Å, 5 µm, 20 x 3.9 mm) and a Resolve C18, 90Å, 5 µm column (150 mm × 3.9 mm i.d.) from Waters (MA, USA). A mobile phase of methanol-acetonitrile was used in proportion 98:2 (v/v). The wavelength used for the detection of α-tocopherol was 292 nm.

Sensory evaluation: A representative part of seed from each of the culture systems was subjected to a popping process by heating over a burning clay pot, following a traditional method. The seed was subjected to a sensory evaluation using the duo-trio test with balanced references that is 50% of the tests were conducted using popped seed from greenhouse and 50% of the tests used popped seed from the open field. The samples were tested by 120 untrained college panelists who had not consumed food or beverages (except natural water) at least 2 h before the test (Kim and Lee, 2012).

Statistical Analysis

Descriptive statistics were used to define the behavior of the variables studied. To compare the differences in the variables studied between the two cultivation systems (greenhouse *vs*. open field) Student's *t* test was used. An analysis of variance (ANOVA) followed by *post hoc* Tukey test was used to analyze possible changes in the variables over time (48, 58 and 76 days). A confidence interval of 95% and a level of significance ($p<0.05$) were performed using SPSS V23 for Windows.

Results

Temperature and Humidity

The thermal environment recorded from March to July was different in the two cultivation systems (Table 1). As expected, higher average values for temperature and humidity were observed in the greenhouse compared to those recorded in the open field. The minimum and maximum values of temperature and humidity varied greatly in both cultivation systems, which is typical for this arid region in Querétaro, where there is a strong climatic

contrast between day and night. Average temperature in the greenhouse from March to July was 21.8 to 25.2°C, with highs of 46.5 to 52.0°C. In contrast, average in the open field was 17.9 to 23.4°C, with highs of 25 to 29°C.

Anti-nutritional Factors

Phytic acid: According to the data obtained from the samples of greenhouse cultivation, the content of phytic acid was slightly higher than that of the open field in 2.5, 3.7 and 1.9% in amaranth leaves at 48, 58 and 76 days of culture, but these differences were not significant ($p<0.05$). A higher phytic acid content (36.2%) was found in the seed from the open field crop (Table 2).

Trypsin inhibitory activity: The values of pure trypsin inhibited in *A. hypochondriacus* samples remained stable throughout the crop cycle in both systems in the leaf samples of amaranth. The values of pure trypsin inhibited were higher ($p<0.05$) in samples from open field cultivation, although statistically different, the values were very close. Also, no variations were observed over the length of the crop cycle, and values were stable across different sampling points (at 48, 58 and 76 days). As for the amaranth seed, a significant increase was observed in the open field samples (14.14 mg of pure trypsin inhibited/g dry matter), this value being five times more than the greenhouse samples (Table 2).

Antioxidant Capacity

ABTS: The results obtained from testing for antioxidant capacity using the ABTS method show higher values ($p<0.05$) in the three samples of amaranth leaf cultivation in open field, of 2.2, 3.1 and 1.4 times at 48, 58 and 76 days, respectively. They also showed a decrease in the antioxidant capacity of the leaf at 58 days in greenhouse cultivation, while antioxidant capacity of open field cultivation is shown to decrease noticeably in the leaf over time (Table 2).

FRAP: According to the FRAP method, the values for antioxidant capacity in the three samplings were higher ($p<0.05$) in plants grown in the open field by 58, 53 and 50% at 48, 58 and 76 days, respectively. In samples of seed amaranth, there was no significant difference between the two cultivation systems (Table 3). The antioxidant capacity measured by the FRAP method was stable over time in leaf samples from both greenhouse and open field cultivation.

DPPH: The results obtained for antioxidant capacity using the DPPH method showed higher values ($p<0.05$) in all leaf samples from the open field system, an additional 26, 30 and 64% at 48, 58 and 76 days, respectively. Also, the seed from open field exhibited greater antioxidant capacity, i.e., 2.9 times higher than the seed from greenhouse cultivation (Table 3).

Phenolic Compounds

Total phenolic compounds: The amount of total phenolic compounds was higher in Amaranth leaf samples in the open field by 74, 64 and 59% at 48, 58 and 76 days of cultivation, respectively. With regard to amaranth seed, phenolic compounds in open field were 5 times higher than in the greenhouse. In both cropping systems, at 58 days after the start of cultivation, an increase in the content of total phenolic compounds was observed (Table 4).

Total anthocyanins: The amount of total anthocyanins in the leaf samples from the greenhouse declined gradually starting at 48 day (896 mg/100 g DM) and up to day 76 (708.80 mg/100 g DM), with a reduction of 20.9%. In contrast, the amount of anthocyanins in leaf samples from the open field varied. It increased from their initial value of 352 mg/100 g DM at 48 days to 743 mg/100 g DM at 58 days, then falling again to 376.04 mg/100 g DM at 76 days (Table 5). In all cases, the content of anthocyanins in the leaf samples grown in the greenhouse was higher than those grown in the open field. For seed amaranth, the opposite effect was observed, with total anthocyanins 1.85 times higher in greenhouse amaranth seeds than in the open field samples.

Condensed tannins: Condensed tannin content is shown in Table 6. This shows that the values from the first sampling point (48 days) are superior in open-field cultivation, but not significantly ($p>0.05$). In contrast, at the second sampling point (58 days), in leaf amaranth, changes occur in both cropping systems that match the period of inflorescence of the amaranth plant: while in samples grown in the greenhouse, a decrease of 8.28% was observed (from 4612 to 4229 mg catechin equivalents/100 g DM), and in samples from the open field, an increase of 31% was observed in the second sampling (from 5426 to 7106 mg catechin equivalents/100 g DM: Table 6). Finally, the contents of condensed tannins at 76 days after seeding were similar in both of the cropping systems.

α-tocopherol

According to the results obtained from measuring α-tocopherol in the amaranth samples grown in the greenhouse, the values were higher in the three leaf cuttings by 26, 46.9 and 53% compared to the open field crop. In the case of seed, the reading was higher in open field cultivation by 26.9%.

Sensory Evaluation

Total of 120 duo-trio tests were divided into 4 non-consecutive days, obtaining 66 hits (55%) and 54 failures (45%). A minimum number of correct answers was evaluated to establish significance at different probability levels according to a binomial distribution. The results obtained in the duo-trio test indicate that no sensory differences were found between the popped amaranth seeds from greenhouse or open field. There was no evident sensory detection of differences in nutrition and composition between the two cultivation systems.

Table 1: Average temperature and humidity recorded in the two amaranth cultivation systems

Temperature (°C)	Greenhouse (mean ± sd)	(min-max)	Open field(mean ± sd)	(min-max)
March	21.8 ± 12.2	11.2 - 46.5	17.9 ± 5.5	6.9 - 26.3
April	28.9 ± 08.8	8.9 - 52.0	18.6 ± 6.1	5.1 - 28.9
May	23.5 ± 10.3	9.8 - 48.2	23.1 ± 3.4	8.7 - 28.3
June	24.3 ± 13.5	13.6 - 41.5	18.7 ± 5.3	10.7 - 29.0
July	25.2 ± 11.9	11.9 - 47.6	23.4 ± 6.3	9.0 - 25.0
Humidity(%)				
March	52.5 ± 23.4	14.4 – 86.0	50.6 ± 24.7	13.0 – 94.1
April	47.8 ± 22.5	8.4 – 83.0	44.8 ± 25.3	5.0 – 95.3
May	52.6 ± 18.3	15.0 – 78.4	40.4 ± 15.3	11.2 – 65.1
June	52.3 ± 17.4	16.4 – 81.3	50.7 ± 21.5	12.0 – 89.7
July	51.8 ± 15.5	17.6 – 88.0	46.1 ± 24.6	7.0 – 80.3

Table 2: Phytic acid and trypsin inhibited contents in samples of amaranth leaf and seed

Sampled part (time of sampling)	Greenhouse	Open field
	Phytic acid (sodium phytate g/100 g dry matter)	
Leaf (48 days)	4.99 ± 0.1[a]	4.87 ± 0.1[a]
Leaf (58 days)	5.04 ± 0.1[a]	4.86 ± 0.1[a]
Leaf (76 days)	4.92 ± 0.1[a]	4.83 ± 0.1[a]
Seed	1.20 ± 0.0	1.88 ± 0.0*
	Trypsin inhibited (mg of pure trypsin inhibited/g dry matter)	
Leaf (48 days)	2.28 ± 0.1[a]	2.52 ± 0.1[a]*
Leaf (58 days)	2.43 ± 0.0[a]	2.59 ± 0.0[a]*
Leaf (76 days)	2.19 ± 0.0[a]	2.53 ± 0.1[a]*
Seed	2.78 ± 0.1	14.14 ± 0.1*

Results are expressed as the average of 3 determinations ± 1 standard deviation
*Indicates a significant difference ($p<0.05$) in the same variable when comparing greenhouse vs. open field. Different letters indicate a significant difference ($p<0.05$) comparing different leaf samples times from each cultivation system

Table 3: Antioxidant capacity in samples of amaranth leaf and seed measured by the ABTS, FRAP and DPPH methods

Sampled part (time of sampling)	Greenhouse	Open field
	ABTS (mg ascorbic acid equivalents/100 g dry matter)	
Leaf (48 days)	93.25 ± 3.6[a]	207.51 ± 13.0[a]*
Leaf (58 days)	60.47 ± 1.6[b]	186.06 ± 17.2[a]*
Leaf (76 days)	90.73 ± 0.9[a]	126.95 ± 2.6[b]*
Seed	ND	ND
	FRAP (g ascorbic acid equivalents/100 g dry matter)	
Leaf (48 days)	5.28 ± 0.2[a]	8.35 ± 0.2[a]*
Leaf (58 days)	5.05 ± 0.1[a]	7.75 ± 0.2[a]*
Leaf (76 days)	5.15 ± 0.1[a]	7.71 ± 0.3[a]*
Seed	0.61 ± 0.0	0.64 ± 0.0
	DPPH (mg ascorbic acid equivalents/100 g dry matter)	
Leaf (48 days)	282.21 ± 8.2[a]	356.54 ± 14.6[a]*
Leaf (58 days)	226.56 ± 5.5[b]	293.98 ± 6.3[b]*
Leaf (76 days)	244.78 ± 2.0[b]	401.43 ± 0.5[a]*
Seed	53.37 ± 13.9	153.9 ± 10.0*

Results are expressed as the average of 3 determinations ± 1 standard deviation
*Indicates a significant difference ($p<0.05$) in the same variable when comparing greenhouse vs. open field. Different letters indicate a significant difference ($p<0.05$) comparing different leaf samples times from each cultivation system

Discussion

Phytic acid is the main anti-nutrient present in cereals. It is known to be present in high concentrations in cereal seeds and legumes, and it may significantly inhibit mineral absorption by forming insoluble complexes with minerals, such as non-heme iron, calcium and zinc; thus compromising the performance of proteins (Hurrell *et al.*, 1992; Bohn *et al.*, 2004). The amount of phytic acid in leaves of amaranth remained constant throughout the life cycle of the plant. Phytic acid values were higher in this species than in other seeds like maize (whole seed) 0.90, rice (polished) 0.13, rye (whole seed) 0.68, soybean (dehulled and toasted) 0.90, and wheat (whole seed) 0.89 mg/g (Egli *et al.*, 2003), but are within the range of values (from 0.52 to 2.24 g/100 g) reported for different amaranth species (Lorenz and Wright, 1984; Pedersen *et al.*, 1987; Amare *et al.*, 2016). Trypsin is an enzyme present in the digestion of proteins. The inhibitor of this enzyme reduces its biological activity. Therefore, from the point of view of consumption, protease inhibitors that interfere with the activity of this enzyme can be regarded as anti-nutritional factors (Savage and Morrison, 2003). According to the results of this study, the content of trypsin inhibitors may be reduced considerably by greenhouse cultivation, when compared to the cultivation of amaranth in an open field. Nagamatsu López *et al.* (2004) suggested that increase of trypsin inhibitors in the leaves of plants exposed to direct sunlight in amaranthus may have a protective function against the damage caused by strong sunlight.

Table 4: Content of total phenolic compounds in samples of amaranth leaf and seed

Sampled part (time of sampling)	Total phenolics (mg gallic acid equivalents/100 g dry matter)	
	Greenhouse	Open field
Leaf (48 days)	1311.70 ± 9.6[a]	2279.95 ± 89.0[a]*
Leaf (58 days)	1621.04 ± 93.5[b]	2656.87 ± 66.0[b]*
Leaf (76 days)	1405.03 ± 48.9[a]	2238.27 ± 51.6[a]*
Seed	5.47 ± 2.1	27.36 ± 5.3*

Results are expressed as the average of 3 determinations ± 1 standard deviation
*Indicates a significant difference ($p<0.05$) in the same variable when comparing greenhouse vs. open field. Different letters indicate a significant difference ($p<0.05$) comparing different leaf samples times from each cultivation system

Table 5: Total anthocyanin content in samples of amaranth leaf and seed

Sampled part (time of sampling)	Greenhouse	Open field
	Anthocyanins (mg equivalents of cyanidin 3-glucoside/100 g dry matter)	
Leaf (48 days)	896.02 ± 6.2[a]	352.21 ± 21.9[a]*
Leaf (58 days)	818.26 ± 33.6[b]	742.97 ± 12.3[b]*
Leaf (76 days)	708.80 ± 12.4[c]	376.04 ± 18.8[a]*
Seed	40.94 ± 0.8	74.39 ± 1.6*

Results are expressed as the average of 3 determinations ± 1 standard deviation
*Indicates a significant difference ($p<0.05$) in the same variable when comparing greenhouse vs. open field. Different letters indicate a significant difference ($p<0.05$) comparing different leaf samples times from each cultivation system

Table 6: Condensed tannin content in samples of amaranth leaf and seed

Sampled part (time of sampling)	Greenhouse	Open field
	Condensed tannins (mg catechin equivalents/100 g dry matter)	
Leaf (48 days)	4611.61 ± 185.8[a]	5426.03 ± 364.4[a]
Leaf (58 days)	4229.68 ± 166.9[b]	7106.21 ± 495.5[b]*
Leaf (76 days)	5442.81 ± 387.6[a]	5243.19 ± 251.8[a]
Seed	2242.68 ± 68.0	5366.06 ± 425.8*

Results are expressed as the average of 3 determinations ± 1 standard deviation
* Indicates a significant difference ($p<0.05$) in the same variable when comparing greenhouse vs. open field. Different letters indicate a significant difference ($p<0.05$) comparing different leaf samples times from each cultivation system

Table 7: α-tocopherol content in samples of amaranth leaf and seed

Sampled part (time of sampling)	Greenhouse	Open field
	(mg α-tocopherol/100 g dry matter)	
Leaf (48 days)	63.8 ± 1.1[a]	47.2 ± 1.3[a*]
Leaf (58 days)	41.6 ± 1.7[b]	22.1 ± 1.2[b]*
Leaf (76 days)	51.0 ± 0.5[c]	27.4 ± 1.9[c*]
Seed	1.4 ± 0.1	1.7 ± 0.0

Results are expressed as the average of 3 determinations ± 1 standard deviation
*Indicates a significant difference ($p<0.05$) in the same variable when comparing greenhouse vs. open field. Different letters indicate a significant difference ($p<0.05$) comparing different leaf samples times from each cultivation system

The increase of trypsin inhibitors in leaves of amaranth exposed to direct sunlight may have been part of a defense system against the damaging effects of ultraviolet (UV) light levels found in strong solar radiation (Conconi *et al.*, 1996). Perhaps the greatest ABTS values of antioxidant capacity observed in open field cultivation are due to responses to environmental conditions to which the plants were subjected during the cultivation period, for example exposure to direct sunlight (Conconi *et al.*, 1996). In greenhouse cultivation, average temperatures were higher than those recorded in the open field, oscillating between 21.8–25.2ºC and 17.9–23.4ºC, respectively, during the cultivation cycle (Table 1). The maximum temperature recorded was in the month of April, when it was 47% higher in the greenhouse (52ºC) than in the open field (where the maximum temperature was 28.9ºC). In April (month with highest temperature), when the first leaf cut was performed, α-tocopherol values were the highest, in comparison with the other leaf cuts throughout the crop cycle (Table 7). This may be because α-tocopherol content is strongly influenced by different stress conditions, one of them being high temperatures. The high temperatures modifies the production of secondary metabolites, the reason some authors propose α-tocopherol as an indicator of stress resistance (Mahajan and Tuteja, 2005; Wahid, 2007; Foyer and Noctor, 2009). The values of α-tocopherol obtained in seeds coincide with those reported in amaranth seed in a range of 0.297 to 1.565 mg/100 g (Kraujalis *et al.*, 2013).

The methods for determining antioxidant activity verify that an oxidizing agent induces oxidative damage to

an oxidizable substrate, and this oxidation is inhibited or reduced in the presence of an antioxidant. This inhibition is proportional to the antioxidant activity of the compound or sample. Different techniques are used to acquire a more complete antioxidant profile (Boligon *et al.*, 2014). The ABTS, FRAP and DPPH methods, being simple, rapid and reproducible, can complement each other in the study of the antioxidant capacity of a sample (Chlopicka *et al.*, 2012). In this study, we observed a strong correlation between the different antioxidant capacity methods: DPPH and FRAP ($r= 0.931$, $p<0.01$), DPPH and ABTS ($r=0.863$, $p<0.01$), FRAP and ABTS ($r=0.969$, $p<0.01$). According to these three tests measuring antioxidant capacity in leaf and seed amaranth, the samples from open field showed higher antioxidant capacity, possibly because they experienced greater exposure to direct sunlight. In a study on quantification of polyphenols and antioxidant activity in amaranth and quinoa sprouts, although statistically insignificant, polyphenol content was higher in sprouts grown in light compared to those grown in the dark (Pasko *et al.*, 2009). In another study, plants growing under shade conditions produced significantly fewer phenolics compared with those growing in full light (Hofland-Zijlstra and Berendse, 2009). This study found a positive correlation between the content of total phenolic compounds and antioxidant capacity: DPPH ($r=.709$, $p<0. 01$), FRAP ($r= 0.724$, $p<0.01$) and ABTS (0.746, $p<0.01$). In addition to phenolic compounds, antioxidant capacity may be related to other compounds such as amino acids, peptides or squalene (Gamel *et al.*, 2007; Gorinstein *et al.*, 2007; Rivera *et al.*, 2010; Tironi and Añón, 2010) and likewise to other antioxidant compounds present in the extracts such as vitamins, fatty acids and lipid derivatives (López-Mejía *et al.*, 2014). In the present study, both the antioxidant capacity and total phenolic compounds were higher in leaves and seeds samples from the open field cultivation (Table 3 and 4).

The variation in total anthocyanins in leaf amaranth samples from open field cultivation was similar to that reported at maturity stages for maize plant (Mendoza, 2012) and camu-camu shell (Villanueva-Tiburcio *et al.*, 2010). In the present study, the values of anthocyanins in amaranth leaf from the open field cultivation system were lower at 76 days after planting, while values in seed amaranth were higher; compared to the values obtained from greenhouse seed (Table 5). Anthocyanins are phenolics and are responsible for pigmentation of the tissues that produce them. One study found that an increase in anthocyanins present in amaranth and quinoa sprouts was due to the effect of daylight and sprout days (Taylor and Briggs, 1990). Sprouts grown under daylight had higher anthocyanins contents than those grown in the dark. The authors mention that this effect is controlled by multiple regulatory genes, and that is induced by various factors, for example sunlight. In contrast, in our study, amaranth leaves grown in the greenhouse presented higher anthocyanin content than to those grown in open field, which received direct sunlight (Table 5). In addition to light, other factors such as temperature or ionic stress are involved in anthocyanin synthesis (Dube *et al.*, 1992; Sène *et al.*, 2001; Wahid, 2007). This means that temperature may be responsible for higher anthocyanins in the leaves of amaranth grown in the greenhouse. This notion is augmented by the fact that the temperature was higher in the greenhouse than in the open field (Table 1), causing a greater synthesis of anthocyanins in the leaves of amaranth grown in greenhouses, than in the open field.

The content of condensed tannins in amaranth seeds obtained from open field cultivation was significantly higher (2.4 times) than in seeds from the greenhouse (Table 6). It is reported that the concentration of total phenolics and condensed tannins was higher in leaves of *Calluna vulgaris* when grown in poor soil humus, than those grown in rich humus (Hofland-Zijlstra and Berendse, 2009). Hansen *et al.* (2006) showed that a combination of nitrogen addition with an increase in air temperature seems to induce a positive response of condensed tannins in leaves more than one year old *Vacciniumvitis-Ideae* and *Cassiopetetragona* but observed no detectable effect on levels of tannins in a shaded environment. In a study on the supply of nutrients and light intensity in heathland ecosystems, the authors conclude that there is a great deal of natural variation in total phenolics and condensed tannins within and between plants, and that this depends largely on the characteristics of place (light, soil nutrients) and plant species (Hofland-Zijlstra and Berendse, 2009). This could explain the results of our study, which involve higher exposure to sunlight and environmental factors in the open field cultivation system. The tannins in plants play a defensive role against consumption by their predators. They deter the growth and survival of many herbivores (Barbehenn and Peter Constabel, 2011). This could explain an increase in condensed tannins at 58 days after sowing in the open field, where amaranth plants were exposed to insects and other organisms, compared to plants grown under greenhouse. It is mentioned that no pesticides were used in either cultivation system.

Conclusion

In general, both antioxidant capacity and secondary metabolites were higher in the cultivation of amaranth in the open field; except that anthocyanins and α-tocopherol were found to be higher in the greenhouse cultivation. The anti-nutritional factors in amaranth seeds were higher in the open field than in the greenhouse. Greenhouse cultivation can offer numerous advantages for production, but in most of the variables, analyzed in this study, it resulted in a disadvantage for field crops. It is necessary to control variables such as the temperature inside the greenhouse and to evaluate the use of elicitors that can be applied inside the greenhouse in order to promote an improvement in the

nutritional composition and the production of antioxidants, as secondary metabolites of plants.

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References

Abdel, E.S. and P.A. Hucl, 1999. A rapid method for quantifyin total anthocyanins in blue aleurone and purple pericarp wheats. *Cereal Chem.*, 76: 350–354

Amare, E., C. Mouquet-Rivier, I. Rochette, A. Adish and G.D. Haki, 2016. Effect of popping and fermentation on proximate composition, minerals and absorption inhibitors, and mineral bioavailability of *Amaranthus caudatus* grain cultivated in Ethiopia. *J. Food. Sci. Technol.*, 53: 2987–2994

Barbehenn, R.V. and C. Peter Constabel, 2011. Tannins in plant-herbivore interactions. *Phytochemistry*, 72: 1551–1565

Benzie, I.F. and J.J. Strain, 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem*., 239: 70–76

Bohn, T., L. Davidsson, T. Walczyk and R.F. Hurrell, 2004. Phytic acid added to white-wheat bread inhibits fractional apparent magnesium absorption in humans. *Am. J. Clin. Nutr.*, 79: 418–423

Boligon, A.A., M.M. Machado and M.L. Athayde, 2014. Technical Evaluation of Antioxidant Activity. *Med. Chem.*, 4: 517–522

Brand-Williams, W., M. Cuvelier and C. Berset, 1995. Use of a free radical method to evaluate antioxidant activity. *Lebensm.-Wiss. Technol.-Food Sci. Technol*., 28: 25–30

Breene, W.M., 1991. Food Uses of Grain Amaranth. *Cereal Foods World*, 36: 426–430

Bressani, R., 2003. Amaranth. *In: Encyclopedia of Food Sciences and Nutrition*, pp: 166–173. Trugo, L. and P.M. Finglas (eds.). Academic Press, Oxford, UK

Chávez-Servín, J.L., H.F. Cabrera-Baeza, E.A. Jiménez-Ugalde, A. Mercado-Luna, K. de la Torre-Carbot, K. Escobar-García, A. Aguilera, J. Serrano-Arellano and T. García-Gasca, 2017. Comparison of chemical composition and growth of amaranth (*Amaranthus hypochondriacus*) between greenhouse and open field systems. *Int. J. Agric. Biol*., 19: 577–583

Chlopicka, J., P. Pasko, S. Gorinstein, A. Jedryas and P. Zagrodzki, 2012. Total phenolic and total flavonoid content, antioxidant activity and sensory evaluation of pseudocereal breads. *Lebensm.-Wiss. Technol.-Food Sci. Technol*., 46: 548–555

Conconi, A., M.J. Smerdon, G.A. Howe and C.A. Ryan, 1996. The octadecanoid signalling pathway in plants mediates a response to ultraviolet radiation. *Nature*, 383: 826–829

Corke, H., Y.Z. Cai and H.X. Wu, 2016. Amaranth: Overview. *In: Reference Module in Food Science*, pp: 1–6. Smithers, G.W. (ed.). Elsevier, Amsterdam, Netherlands

Deshpande, S.S. and M. Cheryan, 1985. Evaluation of vainillin assay for tannin analysis of dry beans. *J. Food. Sci.*, 50: 905–910

Dube, A., S. Bharti and M.M. Laloraya, 1992. Inhibition of anthocyanin synthesis by cobaltous ions in the first internode of *Sorghum bicolor* L. Moench. *J. Exp. Bot.*, 43: 1379–1382

Egli, I., L. Davidsson, M.A. Juillerat, D. Barclay and R. Hurrell, 2003. Phytic acid degradation in complementary foods using phytase naturally occurring in whole grain cereals. *J. Food. Sci*., 68: 1855–1859

Fidantsi, A. and G. Doxastakis, 2001. Emulsifying and foaming properties of amaranth seed protein isolates. *Colloids Surf. B-Biointerfaces*, 21: 119–124

Foyer, C.H. and G. Noctor, 2009. Redox regulation in photosynthetic organisms: signaling, acclimation, and practical implications. *Antioxid. Redox. Signal*., 11: 861–905

Fuller, R. and A. Zahnd, 2012. Solar Greenhouse technology for food security: a case study from Humla District, NW Nepal. *Mt. Res. Dev.*, 32: 411–419

Gamel, T., A. Mesallam, A. Damir, L. Shekib and J. Linssen, 2007. Characterization of amaranth seed oils. *J. Food Lip.*, 14: 323–334

Gao, Y., C. Shang, M.A. Saghai Maroof, R.M. Biyashev, E.A. Grabau, P. Kwanyuen, J.W. Burton and G.R. Buss, 2007. A Modified colorimetric method for phytic acid analysis in soybean. *Crop Sci*., 47: 1797–1803

Gimeno, E., A.I. Castellote, R.M. Lamuela-Raventós, M.C. de la Torre and M.C. López-Sabater, 2000. Rapid determination of vitamin E in vegetable oils by reversed-phase high-performance liquid chromatography. *J. Chromatogr. A*, 881: 251–254

Gorinstein, S., O.J.M. Vargas, N.O. Jaramillo, I.A. Salas, A.L.M. Ayala, P. Arancibia-Avila, F. Toledo, E. Katrich and S. Trakhtenberg, 2007. The total polyphenols and the antioxidant potentials of some selected cereals and pseudocereals. *Europ. Food Res. Technol*., 225: 321–328

Hansen, A.H., S. Jonasson, A. Michelsen and R. Julkunen-Tiitto, 2006. Long-term experimental warming, shading and nutrient addition affect the concentration of phenolic compounds in arctic-alpine deciduous and evergreen dwarf shrubs. *Oecologia*, 147: 1–11

Hofland-Zijlstra, J.D. and F. Berendse, 2009. The effect of nutrient supply and light intensity on tannins and mycorrhizal colonisation in Dutch heathland ecosystems. *Plant Ecol*., 201: 661–675

Hurrell, R.F., M.A. Juillerat, M.B. Reddy, S.R. Lynch, S.A. Dassenko and J.D. Cook, 1992. Soy protein, phytate, and iron absorption in humans. *Am. J. Clin. Nutr*., 56: 573–578

Khandaker, L., A.S.M.G. Akond, M. Ali and S. Oba, 2010. Biomass yield and accumulations of bioactive compounds in red amaranth (*Amaranthus tricolor* L.) grown under different colored shade polyethylene in spring season. *Sci. Hortic*., 123: 289–294

Kim, M.A. and H.S. Lee, 2012. Investigation of operationally more powerful duo-trio test protocols: Effects of different reference schemes. *Food. Qual. Prefer*., 25: 183–191

Kraujalis, P., P.R. Venskutonis, V. Kraujaliene and A. Pukalskas, 2013. Antioxidant properties and preliminary evaluation of phytochemical composition of different anatomical parts of amaranth. *Plant Foods Hum. Nutr.*, 68: 322–328

Liener, I.E., 1977. Protease inhibitors and other toxic factors in seeds. *In: Plant Proteins*. pp: 117–140. G. Norton (ed.). Butterworth-Heinemann

López-Mejía, A., A. López-Malo and E. Palou, 2014. Antioxidant capacity of extracts from amaranth (*Amaranthus hypochondriacus* L.) seeds or leaves. *Ind. Crops Prod*., 53: 55–59

Lorenz, K. and B. Wright, 1984. Phytate and tannin content of amaranth. *Food Chem.*, 14: 27–34

Luedtke, A.N., B. Chapman and D.A. Powell, 2003. Implementation and analysis of an on-farm food safety program for the production of greenhouse vegetables. *J. Food Prot.*, 66: 485–489

Mahajan, S. and N. Tuteja, 2005. Cold, salinity and drought stresses: an overview. *Arch. Biochem. Biophys*., 444: 139–158

Mendoza, C.G., 2012. Las antocianinas del maíz: Su distribución en la planta y producción. Available at: https://es.scribd.com/document/218949483/Mendoza-Mendoza-CG-MC-Genetica-2012 (Accessed: 12 April 2018)

Morales, J., N. Vásquez, and R. Bressani, 2009. *El Amaranto, características físicas, químicas, toxicológicas y funcionales y aporte nutricio*, 1st edition. Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, DF, Mexico

Nagamatsu López, Y., A. Blanco Labra, J. Délano Frier, and E. Pimienta Barrios, 2004. Light intensity and activity of trypsin inhibitors in amaranth leaves and seeds. *Rev. Fitotec. Mex.*, 27: 127–132

Orona-Tamayo, D. and O. Paredes-López, 2017. Chapter 15 – Amaranth part 1—sustainable crop for the 21st century: Food properties and nutraceuticals for improving human health. *In: Sustainable Protein Sources*, pp: 239–256. J.P.D. Wanasundara and L. Scanlin (eds.). Academic Press, San Diego, USA

Pasko, P., H. Barton, P. Zagrodzki, S. Gorinstein, M. Folta and Z. Zachwieja, 2009. Anthocyanins, total polyphenols and antioxidant activity in amaranth and quinoa seeds and sprouts during their growth. *Food Chem.*, 115: 994–998

Pedersen, B., L.S. Kalinowski and B.O. Eggum, 1987. The nutritive value of amaranth grain (Amaranthus caudatus). *Plant Foods Hum. Nutr.*, 36: 309–324

Pospisil, A., M. Pospisil, B. Varga and Z. Svecnjak, 2006. Grain yield and protein concentration of two amaranth species (*Amaranthus* spp.) as influenced by the nitrogen fertilization. *Europ. J. Agron.*, 25: 250–253

Ramos-Díaz, J.M., S. Kirjoranta, S. Tenitz, P.A. Penttila, R. Serimaa, A.M. Lampi and K. Jouppila, 2013. Use of amaranth, quinoa and kaniwa in extruded corn-based snacks. *J. Cereal. Sci.*, 58: 59–67

Rivera, G., V. Bocanegra-García and A. Monge, 2010. Traditional plants as source of functional foods: a review. *CyTA-J. Food*, 8: 159–167

Sarmadi, B., Y. Rouzbehan and J. Rezaei, 2016. Influences of growth stage and nitrogen fertilizer on chemical composition, phenolics, in situ degradability and in vitro ruminal variables in amaranth forage. *Anim. Feed Sci. Technol*., 215: 73–84

Savage, G.P. and S.C. Morrison, 2003. Trypsin inhibitors. *In Encyclopedia of Food Sciences and Nutrition*. pp: 5878–5884. Trugo and P.M. Finglas (eds.). Academic Press, Oxford, UK

Sène, M., T. Doré and C. Gallet, 2001. Relationships between biomass and phenolic production in grain sorghum grown under different conditions. *Agron. J*., 93: 49–54

Serrano-Arellano, J., M. Gijón-Rivera, J.L. Chávez-Servín, K. de la Torre-Carbot, J. Xamán, G. Álvarez and J.M. Belman-Flores, 2015. Numerical study of thermal environment of a greenhouse dedicated to amaranth seed cultivation. *Solar Energy*, 120: 536–548

Silverstone, S.E., 1997. Food production and nutrition for the crew during the first 2-year closure of Biosphere 2. *Life Support. Biosph. Sci.*, 4: 167–178

Singleton, S.L. and J.A. Rossi, 1965. Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents. *Amer. J. Enol. Vitic.*, 16: 144–158

Sogbohossou, O.E.D. and E.G. Achigan-Dako, 2014. Phenetic differentiation and use-type delimitation in Amaranthus spp. from worldwide origins. *Sci. Hortic.*, 178: 31–42

Stoknes, K., F. Scholwin, W. Krzesinski, E. Wojciechowska and A. Jasinska, 2016. Efficiency of a novel "Food to waste to food" system including anaerobic digestion of food waste and cultivation of vegetables on digestate in a bubble-insulated greenhouse. *Waste Manage*., 56: 466–476

Taylor, L.P. and W.R. Briggs, 1990. Genetic regulation and photocontrol of anthocyanin accumulation in maize seedlings. *Plant Cell*, 2: 115–127

Tironi, V.A. and M.C. Añón, 2010. Amaranth proteins as a source of antioxidant peptides: Effect of proteolysis. *Food Res. Int.*, 43: 315–322

Vaintraub, I.A. and N.A. Lapteva, 1988. Colorimetric determination of phytate in unpurified extracts of seeds and the products of their processing. *Anal. Biochem*., 175: 227–230

Vargas-Ortiz, E., J.P. Delano-Frier and A. Tiessen, 2015. The tolerance of grain amaranth (*Amaranthus cruentus* L.) to defoliation during vegetative growth is compromised during flowering. *Plant Physiol. Biochem.*, 91: 36–40

Venskutonis, P.R. and P. Kraujalis, 2013. Nutritional components of amaranth seeds and vegetables: A review on composition, properties, and uses. *Compr. Rev. Food. Sci. Food Saf*., 12: 381–412

Villanueva-Tiburcio, J.E., L.A. Condezo-Hoyos and E. Ramírez, 2010. Antocianinas, ácido ascórbico, polifenoles totales y actividad antioxidante en la cáscara de camu-camu (*Myrciaria dubia* (H.B.K.) McVaugh). *Food Sci. Technol*., 30: 151–160

Wahid, A., 2007. Physiological implications of metabolites biosynthesis in net assimilation and heat stress tolerance of sugarcane (*Saccharum officinarum*) sprouts. *J. Plant Res*., 120: 219–228

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