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Abstract In this work, the amino acid and fatty acid profiles were determined in two advanced lines of amaranth seeds: *Amaranthus hypochondriacus* × *Amaranthus cruentus* AH17a and *Amaranthus cruentus* AcG6/17a; as well as in two new varieties: *Amaranthus cruentus* var. *Candil* and *Amaranthus hypochondriacus* var. *Dorado*. The following contents were found: protein (18.76–26.00 %), dietary fiber (15.91–17.80 %) and total lipids (10.62–11.44 %), high concentrations of unsaturated fatty acids (77.80–82 % of total lipids), linoleic acid (41.94–55.50 % of total lipids) and lysine (47.3–68.6 mg g^{-1} of protein) were also found. Based on these composition data, chemometric tools were used to classify these new varieties and lines by unsupervised methods—principal component analysis and cluster analysis; as well as by supervised methods—sequential discriminant analysis (DA) and partial least squares DA. It was possible to correctly classify all varieties and lines using 11 variables. In conclusion, it was found that new varieties and advanced lines of amaranth show proper nutritional quality, which reveals the potential of this genus as agro-food. Also, a complete chemometric assessment allowed us to distinguish between these new varieties and lines.

Keywords *Amaranthus* · Advanced lines · New varieties, amino acids · Fatty acids · Chemometric

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Introduction

Amaranth is a native plant from America, cultivated and used for over 4,000 years. Its seeds were food for the Mayans, Aztecs and Incas, almost as important as corn and beans. Nowadays it is currently being reused for its excellent nutritional quality and wide adaptation, even in unfavorable environments [1].

The nutritional value of amaranth is present in all the plant, especially in the quantity and quality of protein in seeds, which contain about 15 % protein and 60 % starch. Its amino acid profile has become an attractive protein source for its high lysine content. The amaranth seed contains between 5 and 8 % fat and it is recognized as the vegetable source with the highest concentration of squalene. Also important is its contribution in fiber, minerals and vitamins [2, 3].

Due to the characteristics of its grains and because it does not belong to the *Gramineae* genus, amaranth is considered to be a pseudo-cereal. Amaranth is a C4 plant, which involves high photosynthetic efficiency, even in adverse weather conditions. Today it is one of the most

promising plants to help reduce the shortage of food as it is possible to use it as diversified horticultural and forage grainer, and as such, it is considered as a food of the future.

In recent years, varieties of amaranths have been evaluated as a functional food based on the content of flavonoids and phenolic acids, which determine the antioxidant activity that is manifested in a decrease in the incidence of chronic diseases [4, 5]. Potential agents have also been detected that reduce cholesterol levels, such as squalene, dietary fiber, tocotrienols and isoprenoid compounds, among others [6]. It is, as well, an important food source for people affected by the celiac disease because it is naturally gluten-free [7, 8].

Nowadays amaranth is cultivated in many parts of the world, including South and Central America, Africa, India, China and the USA. In Argentina, researchers are working on the development of new varieties, i.e. the "breeding establishment" of populations of individuals of this genus, with favorable characteristics such as high production in harvest, great adaptability, ease of yield, and resistance to parasites, among others. These varieties have been obtained by selection from populations with a high degree of variability from those generated by inter-specific crosses.

The aim of this study was to determine proteins, total lipids, dietary fiber, amino acid and fatty acids profile of two new varieties: *Amaranthus cruentus* var. *Candil* (CC), *Amaranthus hypochondriacus* var. *Dorado* (HD); and assess two advanced lines: *Amaranthus hypochondriacus* × *Amaranthus cruentus* H17a (H17) and *Amaranthus cruentus* G6/17a (G6) to evaluate possible changes that could affect their nutritional quality. Also, a complete chemometric assessment was carried out to identify every new variety and advanced line between each other, using as variables the amino acid and fatty acid profiles. Chemometric methods are widely used for classification purposes. Multivariate classification methods are divided in: unsupervised methods, which include principal components analysis (PCA) and cluster analysis (CA), and supervised methods, which include sequential discriminant analysis (SDA) and partial least square discriminant analysis, PLS-DA [9, 10]. This chemometric methods' approach allows to detect hidden properties in the set of samples that cannot be seen in the original data, which can help to identify the differences within the original data and to obtain groups or families, which may be successfully applied to different types of samples [11–13].

Materials and methods

Sampling

Seeds of new varieties of CC and HD, as well as advanced lines of H17 and G6 were supplied by the Faculty of Agronomy and Veterinary, National University of Río Cuarto, Córdoba, Argentina, and obtained from experimental crops of the 2011 vintage.

Sample pretreatment

Dried seeds were ground in a grain mill (CG-8 Stylo 90W 220 V–50 Hz, China) and sieved through a 200 μm mesh. The flours obtained were kept in closed containers protected from light in a cool and dry environment until analysis.

Chemical analysis

All analyses were carried out using eight replicates. Content of: moisture, ash and protein were obtained by AOAC methods [14], soluble dietary fiber (SDF), non-soluble dietary fiber (NDF) and total dietary fiber (TDF) were obtained following the method reported by Prosky et al. [15, 16]; fatty acids were determined as methyl esters by gas chromatography [17, 18].

For fatty acid analysis, the chromatographic method was used in a Varian chromatograph (Berkeley, NC) with a 10 % SP-2330 packed column and flame ionization detector. Standard solutions of fatty acids were acquired from Sigma (St. Louis, MO).

For the determination of the amino acid composition, the technique followed the AOAC Official Method [14]. Samples were defatted for 6 h with hot petroleum ether and then hydrolyzed with 6 mol L^{-1} HCl solution under vacuum for 24 h. The determination of methionine and cysteine and/or cystine was performed with performic acid oxidation followed by acid hydrolysis. Tryptophan was determined after alkaline hydrolysis with a 4.2 mol L^{-1} NaOH solution and subsequent neutralization with a 6 mol L^{-1} HCl solution. The quantification of three hydrolysates was performed by a Beckman amino acid analyzer. Amino acid standard solutions were provided by Sigma (St. Louis, MO). In all cases, analyses were performed by triplicate.

Statistical and chemometric analysis

Statistical differences between advanced lines and new varieties were tested by one-way analysis of variance, ANOVA [19]. PCA and PLS-DA analysis were performed using The Unscrambler 6.11 software (CAMO AS, Trondheim, Norway). CA and LDA were carried out using the InfoStat software (Cordoba, Argentina).

Results and discussion

A complete analysis was carried out in amaranth seed samples, including:

Table 1 Chemical composition of *Amaranthus cruentus* var. *Candil* (CC), *Amaranthus hypochondriacus* var. *Dorado* (HD), advanced lines of *Amaranthus hypochondriacus* × *A. cruentus* H17a (H17) and *Amaranthus cruentus* G6/17 (G6)

Determination (g/100 g)	CC*	HD*	H17*	G6*
Moisture	12.73 ± 0.23^{a}	9.96 ± 0.05^{b}	12.26 ± 0.18^{a}	9.35 ± 0.11^{b}
Ash	4.02 ± 0.19^{a}	3.58 ± 0.22^{a}	3.70 ± 0.18^{a}	3.72 ± 0.18^{a}
Protein ($N \times 6.25$)	12.41 ± 0.01^{a}	15.85 ± 0.21^{b}	12.26 ± 0.18^{a}	14.61 ± 0.08^{c}
Total lipids	9.19 ± 0.27^{a}	7.19 ± 0.17^{b}	9.29 ± 0.29^{a}	9.27 ± 0.41^{a}
SDF	4.03 ± 0.24^{ab}	3.72 ± 0.30^{a}	5.21 ± 0.48^{b}	4.55 ± 0.20^{ab}
NDF	12.58 ± 0.63^{a}	11.98 ± 0.46^{a}	12.60 ± 1.12^{a}	13.13 ± 0.53^{a}
TDF	16.61 ± 0.39^{a}	15.91 ± 0.46^{a}	17.80 ± 1.60^{a}	17.70 ± 0.33^{a}

SDF soluble dietary fiber, *NDF* non-soluble dietary fiber, *TDF* total dietary fiber

* Results are expressed as mean ± standard deviation ($n = 8$). Different letters indicate significant differences ($p < 0.05$)

Table 2 Fatty acids composition (% of total lipids) of seed flour from *Amaranthus cruentus* var. *Candil* (CC), *Amaranthus hypochondriacus* var. *Dorado* (HD), advanced lines *Amaranthus hypochondriacus* × *Amaranthus cruentus* H17a (H17) and *Amaranthus cruentus* G6/17 a (G6)

Carbon atoms	Acid names	CC*	HD*	H17*	G6*
14:0	Myristic	0.20 ± 0.01^{a}	0.20 ± 0.01^{a}	0.20 ± 0.01^{a}	0.20 ± 0.01^{a}
16:0	Palmitic	16.10 ± 0.16^{a}	13.60 ± 0.24^{b}	17.10 ± 0.22^{c}	16.90 ± 0.16^{c}
18:0	Estearic	3.30 ± 0.08^{ac}	2.80 ± 0.04^{b}	3.20 ± 0.13^{a}	3.40 ± 0.16^{c}
20:0	Arachidic	0.80 ± 0.02^{a}	0.60 ± 0.04^{b}	0.70 ± 0.03^{c}	0.80 ± 0.03^{a}
22:0	Behenic	0.30 ± 0.006^{a}	0.30 ± 0.01^{a}	0.30 ± 0.01^{a}	0.30 ± 0.01^{a}
24:0	Lignoceric	0.30 ± 0.01^{a}	0.20 ± 0.01^{b}	0.30 ± 0.01^{a}	0.30 ± 0.01^{a}
16:1	Palmitoleic	0.40 ± 0.03^{a}	0.30 ± 0.01^{b}	0.40 ± 0.01^{a}	0.40 ± 0.01^{a}
18:1n-9	Oleic	30.00 ± 0.70^{a}	22.80 ± 0.40^{b}	28.90 ± 0.66^{a}	32.00 ± 0.50^{c}
18:1n-7	Vaccenic	1.40 ± 0.08^{ab}	1.30 ± 0.07^{b}	1.50 ± 0.05^{ac}	1.60 ± 0.07^{c}
20:1	Gadoleic	0.30 ± 0.01^{a}	0.20 ± 0.01^{b}	0.30 ± 0.01^{a}	0.30 ± 0.01^{a}
18:2	Linoleic	44.87 ± 0.75^{a}	55.50 ± 0.53^{b}	45.14 ± 0.90^{a}	41.94 ± 0.60^{c}
18:3	a-Linolenic	0.70 ± 0.01^{a}	0.70 ± 0.02^{a}	0.80 ± 0.02^{b}	0.70 ± 0.01^{a}
20:4	Arachidonic	1.03 ± 0.03^{a}	1.20 ± 0.05^{b}	0.86 ± 0.01^{c}	0.86 ± 0.02^{c}
Saturated fatty acids		21.00 ± 0.09^{a}	17.70 ± 0.1^{b}	21.70 ± 0.15^{c}	21.90 ± 0.08^{c}
Unsaturated fatty acids		78.70 ± 0.36^{a}	82.00 ± 0.53^{b}	77.90 ± 0.71^{a}	77.80 ± 0.41^{a}
Ratio sat/unsat		0.27	0.21	0.28	0.28

* Results are expressed as mean ± standard deviation ($n = 8$). Different letters indicate significant differences ($p < 0.05$)

- Chemical composition: moisture, ash, protein, SDF, NDF and TDF (Table 1).
- Fatty acids profile: myristic, palmitic, estearic, arachidic, behenic, lignoceric, palmitoleic, oleic, vaccenic, gadoleic, linoleic, a-linolenic and arachidonic acids (Table 2).
- Amino acid profile: alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine + cysteine, phenylalanine + tyrosine, proline, serine, threonine, tryptophan and valine (Table 3).

Table 1 shows the chemical composition (expressed in dry weight) of seed flour: CC, HD and the advanced lines H17 and G6.

The lipid content ranges from 7.19 to 9.29 %, while the protein range varied from 18.76 to 26.00 %: HD showed the highest and H17 the lowest protein values. These values were similar to those reported by Bressani [20] whose protein content range varied from 11.8 to 17.6 % while the lipid ranges varied from 4.8 to 8.1 %.

On the other hand, ranges of TDF (15.91–17.80 %), SDF (3.72–5.21 %) and NDF (11.98–13.13 %) were similar to those reported by Tosi et al. [21], Menegassi et al. [22], and Bressani [20] in different species of amaranth. SDF values were higher than those of cereals and legumes, as for the TDF, no significant differences were found in comparison with most cereals [23]. However, NDF values were lower than those reported for legumes [24]. Epidemiological and

Table 3 Amino acids composition (mg g^{-1} of protein) of seed flour from *Amaranthus cruentus* var. *Candil* (CC), *Amaranthus hypochondriacus* var. *Dorado* (HD), advanced lines *Amaranthus hypochondriacus* × *A. cruentus* H17a (H17) and *Amaranthus cruentus* G6/17 (G6)

Amino acids	CC[a]	HD[a]	H17[a]	G6[a]	FAO[b]
Alanine	48.1 ± 3.3	43.1 ± 3.1	45.2 ± 2.6	47.3 ± 3.5	–
Arginine	109.2 ± 9.7	107.3 ± 8.2	98.5 ± 7.8	91.1 ± 7.5	–
Aspartic acid	114.5 ± 10.1	68.5 ± 4.5	66.3 ± 4.8	125.8 ± 10.5	–
Glutamic acid	171.0 ± 13.2	195.5 ± 15.6	188.3 ± 15.8	221.6 ± 16.7	–
Glycine	82.8 ± 7.3	88.9 ± 6.8	88.7 ± 6.6	87.4 ± 6.9	–
Histidine	40.4 ± 3.2	42.3 ± 3.8	48.8 ± 3.2	40.7 ± 3.1	18
Isoleucine	40.9 ± 2.9	27.8 ± 2.0	29.3 ± 1.8	32.8 ± 2.2	31
Leucine	70.7 ± 5.4	66.7 ± 5.1	69.4 ± 4.4	69.2 ± 5.0	63
Lysine	47.3 ± 3.8	60.3 ± 4.6	68.6 ± 4.8	65.6 ± 4.9	52
Methionine + cysteine	52.3 ± 4.1	54.9 ± 3.4	74.2 ± 5.3	37.2 ± 2.2	26
Phenylalanine + tyrosine	79.2 ± 5.8	66.1 ± 4.6	59.8 ± 5.1	55.8 ± 4.1	46
Proline	58.5 ± 3.4	55.0 ± 3.8	51.2 ± 5.0	53.5 ± 3.6	–
Serine	41.6 ± 3.6	72.3 ± 5.7	60.3 ± 4.9	62.4 ± 5.1	–
Threonine	48.8 ± 3.8	37.7 ± 2.8	44.6 ± 3.8	40.7 ± 3.3	27
Tryptophan	10.4 ± 1.0	14.3 ± 0.9	10.1 ± 0.9	12.1 ± 0.8	7.4
Valine	20.2 ± 1.8	19.2 ± 1.2	16.2 ± 1.1	14.6 ± 0.9	42

[a] Results are expressed as mean ± standard deviation ($n = 8$)

[b] Recommended values by FAO (2007) for children from 1 to 3 years old

clinical studies demonstrate that the consumption of dietary fiber and whole grain intake is inversely related to obesity, diabetes type two, cancer and cardiovascular disease [25]. Given that the recommended value of TDF consumption is 25–36 g day^{-1} (in which 6 g must be SDF), amaranth represents an important contribution to a healthy diet [25].

Table 2 shows the fatty acids' composition of seeds from CC, HD and advanced lines H17 and G6. The importance of knowing the acid profile of these species and of the advanced lines lies in the fact that they may reduce the risk factors for cardiovascular disease, because poor diet with foods rich in saturated and *trans* fatty acids are the most harmful [26]. The total content of unsaturated fatty acids varied from 77.80 to 82.00 %, within which linoleic acid was the main component (41.94–55.50 %) followed by oleic acid (22.80–32.00 %). The total content of saturated fatty acids ranged from 17.70 to 21.90 %, within which palmitic acid was the major component (13.60–17.10 %). Therefore, a significant preponderance of unsaturated fatty acids, and the presence of fatty acids ω3 (alpha linoleic acid), ω6 (linoleic acid) were observed in appropriate proportions that could have positive health benefits and prevent diseases The proportions of saturated and unsaturated acids are consistent with the bibliographic data available for amaranth [27, 28] and the ratio saturated: unsaturated (0.21/0.28) was similar to that reported for wheat, rice bran and soy [28].

The World Health Organization (WHO) recommends the consumption of ω6 and ω3 in a ratio ranging between 5:1 and 10:1 or less [29]. Even though in this work this relationship proved to be higher, the high oleic acid contents act favorably because it is a ω6 fatty acid.

The presence of linoleic acid in appropriate concentration is important, given that it is desirable that the ratio of oleic/linoleic acid should be less than 4, thus limiting the formation of LDL cholesterol [30, 31]; the obtained ratios were 0.41 for HD, 0.64 for H17, 0.67 for CC and 0.76 for G6, which indicate that all plants had lower ratios in comparison to the recommended limit.

The observed variability in the fatty acid profile suggests differences between varieties and between the advanced lines. The ANOVA test detected differences for most of the fatty acids analyzed, except to for the myristic and behenic acids.

Table 3 shows the amino acid composition (mg g^{-1}) for CC, HD, H17 and G6. Considering the essential amino acid composition of the four studied amaranth varieties, all showed a balanced amino acid profile. Lysine (47.3–68.9 mg g^{-1} protein) presented similar values in HD, H17 and G6 amaranth varieties, covering the requirements for all age groups, with the exception of CC (47.3 mg g^{-1} protein) that covers only the requirements established for adults [32]. Levels of histidine (40.4–48.8 mg g^{-1} protein), sulfur amino acids (37.2–74.2 mg g^{-1} protein) and threonine

(37.7–48.8 mg g^{-1} protein), were higher in comparison to those reported by Bressani [20] and Fasuyi [33] for other amaranth species but similar to those found on proteins from cow's milk, beef, and wheat flour. Leucine (66.7–70.7 mg g^{-1} protein) and aromatic amino acids (55.8–79.2 mg g^{-1} protein) showed values similar to other amaranths, but lower than milk and beef; tryptophan (10.1–14.3 mg g^{-1} protein) behaved similarly to other amaranth species and their referenced foods. The content of histidine, leucine, threonine, tryptophan, sulfur and aromatics covered the requirements for all age groups [32]. Isoleucine showed similar values to other amaranth species (27.8–40.9 mg g^{-1} protein): CC and G6 covered the requirements for all age groups, but this did not happen with H17 and HD. Finally, valine (14.6–20.2 mg g^{-1} protein) were found to be below the requirements established for the different age groups [20, 32, 33].

Chemometric analysis

Principal components analysis

The PCA model was obtained by cross-validation, using four PCs, which explained 97.66 % of the original information. The relevant variables were the concentration of the following fatty acids: estearic, arachidic, vaccenic and arachidonic and the amino acids: Arg, Asp, Glu, Met, Lys and Ser. The classification ability of PCA can be seen in Fig. 1 (score plot) where there are four groups which can be distinguished from the others: the CC, G6, HD and H17. Figure 2 shows a loading plot, which explains the influence of the selected variables on the PCA model. As shown, fatty acids presented more influence on PC 1 (mainly estearic acid) with the exception of Asp; while the rest of amino acids had more influence on the PC 2 (mainly on Glu, Lys and Ser). Comparing the score plot (Fig. 1) with

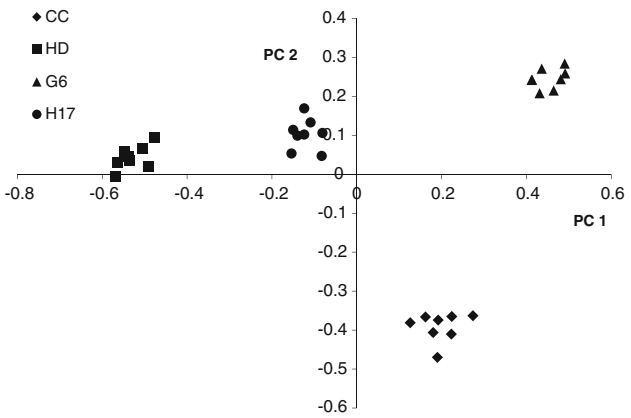

Fig. 1 Score plot of new varieties (CC and HD) and advanced lines (H17 and G6) of amaranth seeds for principal components (PCs) 1 and 2

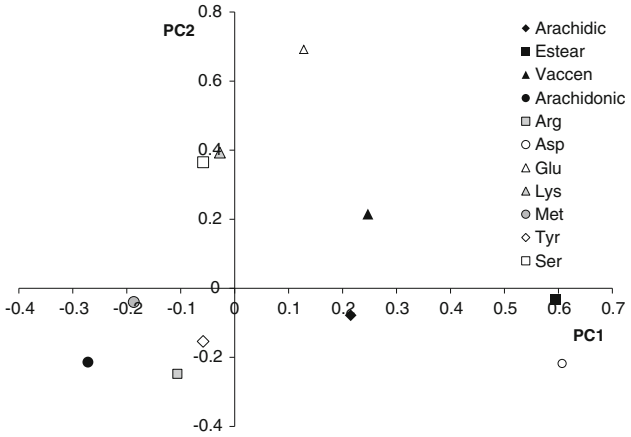

Fig. 2 Loading plot showing the influences of original variables on principal components (PCs) 1 and 2

the loading plot (Fig. 2), it can see the following positives influences: the G6 group is influenced principally by estearic acid, Asp, Lys, Ser and Glu; CC by the estearic acid, Asp and in lesser grade Arg, arachidonic acid and Tyr; H17 by Lys and Ser; HD group by Met, Arg and arachidonic acid.

Cluster analysis

CA was performed using the same variables that were used in the PCA model. The amalgamation criterion was the complete linkage method and the selected distance was the Euclidean square, with which the best results were obtained. Figure 3 shows the dendrogram obtained by CA for the classification of amaranth. Amaranth seed samples were grouped in the four varieties: CC, H17, G6 and HD. Also, the dendrogram shows that the pairs CC–G6 and HD–H17 form two stems, which indicate that there is similarity between pairs. However, this situation cannot be observed in Fig. 1, which adds new information to the multivariate study.

Sequential discriminant analysis

Again, the same original variables used in PCA and CA were used as variables in SDA to confirm the results obtained by means of both previous methods. SDA was used as a supervised method; the classification of amaranth seeds is shown in Table 4. For the training step, six random samples of each variety were used, while for the prediction step, two samples of each one were used. All samples were predicted for the three species in the correct form, without errors in the training and prediction steps. Wilk's λ values for the first and second discriminant functions were 1×10^{-4} and 3×10^{-2}, respectively, with p values <0.05 in both cases, which indicates the good properties of the model.

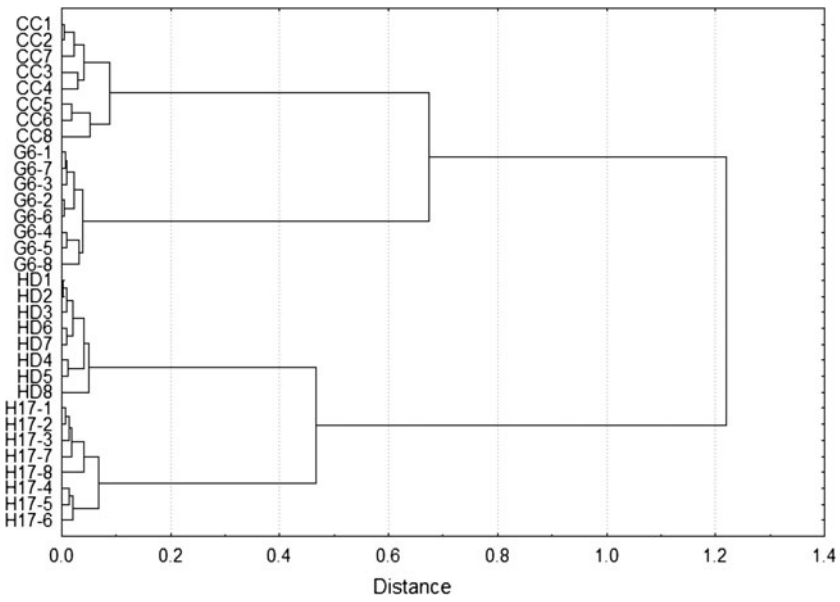

Fig. 3 Dendrogram of cluster analysis of new varieties (CC and HD) and advanced lines (H17 and G6) of amaranth seeds

Table 4 Calibration and prediction set for the sequential discriminant analysis (SDA) model

	CC	HD	G6	H17	Percent of correct
Calibration					
CC	6	0	0	0	100
HD	0	6	0	0	100
G6	0	0	6	0	100
H17	0	0	0	6	100
Prediction					
CC	2	0	0	0	100
HD	0	2	0	0	100
G6	0	0	2	0	100
H17	0	0	0	2	100

Amaranthus cruentus var. *Candil* (CC), *Amaranthus hypochondriacus* var. *Dorado* (HD), advanced lines *Amaranthus hypochondriacus* × *A. cruentus* H17a (H17) and *Amaranthus cruentus* G6/17 (G6)

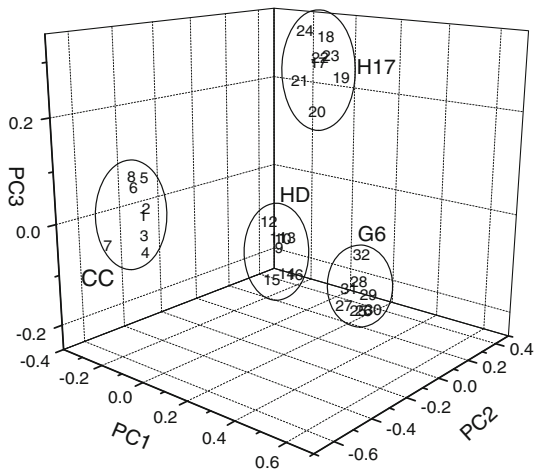

Fig. 4 Classification of new varieties and advanced lines by PLS-DA scores plot

Principal least square discriminant analysis

PLS-DA was performed with the same variables used previously. Unlike the PCA, the PLS-DA model was obtained by cross-validation using three PLS components, which explained 97.95 % of the original variance, with a value of the root mean square of error prediction of 0.026. Twenty samples for the calibration step and eight samples for the prediction set were used to obtain the discriminant table. As with SDA, all samples were correctly classified, with 100 % of correct cases. Then, all samples were included in a PLS-DA scores plot (Fig. 4) which shows a 3D discrimination of the four groups. In this 3D projection, it can see that the G6 (samples 25–32) and HD (samples 9–17) groups are close in comparison to the H17 (samples 18–24) and CC (samples 1–8) groups. On the other hand, the PLS-DA discriminant table (Table 5), showed a 100 % of correct classification in calibration and prediction sets, which is in agree with the results of LDA (Table 4).

The studied amaranth plants showed an interesting content of protein, total lipids, SDF, NDF and TDF. They can be considered a good source of essential amino acids, in agreement with the general approach to the values recommended by FAO/WHO. The comparison of the fatty acid profile of amaranth oil with a conventional cereal such as corn, shows a remarkable similarity and therefore amaranth use can be recommended to be part of the human diet as an alternative source to edible oil.

Based on the results showed in this work, new varieties and advanced lines of amaranth seeds had good nutritional

Table 5 Calibration and prediction set for the partial-least square discriminant analysis (PLS-DA) model

	CC	HD	G6	H17	Percent of correct
Calibration					
CC	6	0	0	0	100
HD	0	6	0	0	100
G6	0	0	6	0	100
H17	0	0	0	6	100
Prediction					
CC	2	0	0	0	100
HD	0	2	0	0	100
G6	0	0	2	0	100
H17	0	0	0	2	100

Amaranthus cruentus var. *Candil* (CC), *Amaranthus hypochondriacus* var. *Dorado* (HD), advanced lines *Amaranthus hypochondriacus* × *A. cruentus* H17a (H17) and *Amaranthus cruentus* G6/17 (G6)

properties, which grant a potential agro-feeding for future uses. On the other hand, chemometric methods showed good ability to distinguish between new varieties and advanced lines of amaranth seeds; therefore, this tools which can be useful in the food industry and international market, as fast methods to classify amaranth seeds.

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