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Productive, Qualitative, and In Vitro Fermentation Traits of Amaranthus Grains as Potential Ingredients for Pig Diet

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Abstract: The present work compared the agronomic traits, chemical composition, fatty acid profile, and in vitro fermentation characteristics of twelve accessions of *Amaranthus* spp., belonging to *A. cruentus*, *A. hybridus*, *A. hypochondriacus*, and *A. tricolor*, grown in a semiarid Mediterranean area. Among accessions, Benin and Arizona (*A. cruentus*) and Pennsylvania (*A. hypochondriacus*) showed the highest seed yield (on average, 322.1 g m^{-2}), while Taiwan (*A. tricolor*) and India and Iowa (*A. hypochondriacus*) the highest thousand seed weight (on average, 0.81 g). Among the species, *A. hypochondriacus* showed the highest crude protein (16 g $100g^{-1}$), starch (51.5 g $100g^{-1}$), and soluble detergent fiber (2.03 g $100g^{-1}$) contents and the most favorable in vitro fermentation characteristics with the highest short-chain fatty acid (SCFA 52.6 mmol g^{-1}) and butyric acid (20.7% SCFA) production together with the lowest crude fiber (4.93 g $100g^{-1}$) and insoluble dietary fiber (12.5 g $100g^{-1}$) content. Arizona (*A. cruentus*) showed the highest level of monounsaturated fatty acids (32.67 g $100g^{-1}$), Ohio (*A. hybridus*) had the highest levels of polyunsaturated fatty acids (44.62 g $100g^{-1}$) and n6-PUFA (44.21 g $100g^{-1}$), and India (*A. hypochondriacus*) had the highest level of n3-PUFA (0.63 g $100g^{-1}$). *A. hypochondriacus* exhibited not only desirable nutritive characteristics, agronomic traits, and suitability to Mediterranean growing conditions, but also a potential beneficial effect. Nonetheless, it is recommended to run longer-term field trials to confirm these findings and to assess the genotype by environment interaction either with current accessions or others from the wide Amaranth germplasm available.

Keywords: pseudo-cereal; accessions; monogastric; quality traits; in vitro degradability; fatty acids profile

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1. Introduction

Amaranth (*Amaranthus* spp.) is a grain crop similar to cereals with desirable agronomic peculiarities and elevated nutritional value suitable for animal nutrition [1]. The genus *Amaranthus* consists of several species, from annual to short-lived perennial plants, and can be grouped into grain and vegetable types. The most important species are *A. cruentus*, *A. hypochondriacus*, *A. hybridus*, and *A. caudatus*, which are mainly used for grain production, while *A. tricolor*, *A. dubius*, and *A. lividus* are used for vegetable production [2]. Recently, grain amaranth has gained popularity as a feed and food crop [3] due to its excellent nutritional value [4]. The protein content is higher (13.0–19.85%) than other cereal grains, with a favorable amino acid profile [5], more lysine than soybean, and relatively abundant sulfur-containing amino acids, which are usually limited in pulse crops [6,7]. Moreover, unlike other cereals, amaranth proteins consist of albumins (about 40%), glutenins (25–30%), and globulins (20%) and contain very small amounts of prolamins (2–3%). Amaranth prolamins

are richer in glutamic acid and essential amino acids than albumins and globulins, which makes amaranth a good protein source closer to the composition of animal proteins [8]. The lipid content (5.5–16.7%) is also higher than conventional cereals [9], and it is characterized by high levels of unsaturated fatty acids [10], with a saturated/unsaturated fatty acid ratio ranging from 0.12 to 0.50 [2,11,12]. Starch is the main component of amaranth grain, although it is slightly lower (48–69%) than in common cereals [2,3]. The total dietary fiber in amaranth grains is slightly lower (7.1–16.4%) than in wheat [2]; nonetheless, dietary fiber has been reported to be comparable to quinoa and other cereals [5,8,13]. Furthermore, the high crude fiber content in amaranth grains has been associated with monogastric animals' gut health [14].

Currently, limited literature exists on the use of amaranth grains in pig nutrition. Zralý et al. [15], tested diets containing 10% amaranth grain on fattening pigs and obtained some remarkable results in growth efficiency, live body weight gains, and health status of pigs during the entire experiment (pre-fattening, fattening stages I and II). Shilov and Zharkovskii [16] reported an increase in digestibility when amaranth was added to pig diets, and Manyelo et al. [3] demonstrated positive results in pig growth performance. Unlike conventional cereals, the proteins and dietary fiber contents in amaranth grains meet the pigs' nutritional requirements [4]. Furthermore, it can be expected that the high content of essential fatty acids could positively modify the lipid composition of the tissues producing healthy meat [17].

A comparison between eight accessions of *Amaranthus cruentus* grown in the Mediterranean area highlighted large genotypic variability of agronomic traits [18], with yields comparable to barley and oat [11]. Taking into account our previous study [19], where *A. cruentus* grains were evaluated as a component of the feeding plan for dairy cows for productive, qualitative, and in vitro fermentation traits, the present study addresses the agronomic traits, proximate composition, in vitro degradability, and fatty acid content of twelve accessions of amaranth, belonging to *A. cruentus*, *A. hypochondriacus*, *A. hybridus*, and *A. tricolor* species, grown in a field trial in a semiarid Mediterranean area. The hypothesis of the investigation is that the nutritional characteristics of amaranth (energy density, protein, starch, and fat content) are useful for pig diets; in fact, the main objective was to explore the quantitative and qualitative profile of amaranth grains as a feed ingredient in swine nutrition.

2. Materials and Methods

2.1. Plant Materials and Field Experiment

The twelve accessions of *Amaranthus* spp. were obtained from the USDA seed bank (Washington, DC, USA), and their main information is reported in Table 1. Accessions were sown in expanded polystyrene trays in a nursery at 26 °C and 85 ± 5% relative humidity (RH) on 21 March 2014 and kept until the fourth true leaf stage.

Meanwhile, a randomized block design with three replications was arranged in a previously plowed and fertilized (40 kg N ha^{-1}, 80 kg P_2O_5 ha^{-1}, and 60 kg K_2O ha^{-1}) sandy–loamy textured soil in Bovalino (20 m a.s.l. 38°08′ N, 16°10′ E, Calabria, Southern Italy). Plants were transplanted at a density of 10 plants m^{-2} in single plots of 9 m^2 (3 × 3 m). Throughout the growing season, weed control was carried out by hand and 320 mm of water was supplied by a drip irrigation system. Before anthesis, a further 80 kg N ha^{-1} was broadcasted. Seed weight was obtained by removing edge plants and by harvesting a sample area of 4 m^2 inside each plot. Due to uneven seed ripening among accessions, grains were harvested at physiological maturity from 29 June to 11 July. Seeds were separated by inflorescences with a laboratory thresher and allowed to dry at room temperature. The mean thousand seed weight was then calculated by three sets of one hundred seeds in each accession and replication. Rainfall amount and distribution and air temperature during the growing season were typical of the area [11].

Table 1. Amaranth (*Amaranthus* spp.) species and origin.

Species	Origin	Accession	Acronym
A. cruentus	Benin (Republic of Benin)	PI 618962	Benin
	Shaba (Kenya)	PI 628793	Shaba
	Arizona (USA)	PI 566896	Arizona
A.hybridus	Delaware (USA)	PI 636181	Delaware
	Ohio (USA)	PI 603891	Ohio
	Goias (Brazil)	PI 652417	Goias
A.hypochondriacus	India	PI 477915	India
	Iowa (USA)	PI 568125	Iowa
	Pennsylvania (USA)	PI 572256	Pennsylvania
A.tricolor	Beijing (China)	PI 419057	Beijing 1
	Taiwan	PI 604669	Taiwan
	Beijing (China)	AMES 26210	Beijing 2

2.2. Sample Preparation

Amaranth seed samples were finely ground with a 1.1 mm sieve by a grounding machine (Pulverisette 19, Fritsch, Idar-Oberstein, Germany).

2.3. Proximate Chemical Analysis

Chemical analyses of the amaranth grain samples were carried out using the standard procedures of the Association of Official Analytical Chemists [20]; method no. 930.15, 942.05, 2001.11, 978.10, and 920.39 for the content of moisture, ash, crude protein, crude fiber, and ether extract were applied.

Total starch content was determined by means of a Megazyme Total Starch Assay Kit (Megazyme©, NEOGEN, Lansing, MI, USA) for enzymatic extraction and using AOAC method no. 996.11 [20]. A UV–visible spectrophotometer setting the wavelength at 510 nm was applied for the quantification of starch content [19].

A Total Dietary Fiber Assay Kit (Megazyme©, NEOGEN, Lansing, MI, USA) for the analysis of total dietary fiber was used.

Each chemical analysis was performed in triplicate for each amaranth accession and the results were expressed as g $100g^{-1}$, as fed.

2.4. Analysis of Fatty Acids, Fatty Acid Classes, and Peroxidation Index Calculation

Lipid extracts underwent direct transesterification for fatty acid (FA) determination [21]. FA methyl esters (FAMEs) were analyzed by a gas chromatograph system (GC-FID, TRACE 1310, Thermo Fisher Scientific, Milan, Italy). For FAME separation, an Omegawax 250 (Supelco, Bellefonte, PA, USA) was used as reported by Oteri et al. [12]. Chromeleon™ Data System Software (Version 7.2.9, Thermo Fisher Scientific, Milan, Italy) was employed for GC-FID data collection. The identification of FA in amaranth seeds was performed by comparing the relative retention times of FAMEs identified in the sample with retention times of the certified standard mixture (mix 37 FAMEs, Supelco, Inc., Bellefonte, PA, USA) analyzed using the same chromatographic method. The single FA concentration was expressed as g $100g^{-1}$ where 100 g was the sum of all areas of FAMEs that were identified. The equation proposed by Luciano et al. [22] was applied to calculate the peroxidation index (PI).

2.5. In Vitro Gas Production

To study the fermentation kinetics in pig's large intestine, the in vitro gas production technique, according to Theodorou et al. [23], was utilized. The twelve amaranth samples were weighed (0.5049 ± 0.0024 g) into 120 mL serum flasks. For each sample, three replications were made and three flasks were incubated without substrate to correct in vitro parameters. Three healthy adult male castrated Large White pigs (mean age:

350 ± 10 days; mean live weight: 159.8 ± 5.2 kg) bred for meat were used as inoculum donor animals. The animals were fed with a commercial diet used for the finishing period (CP: 14.8%; CF: 4.0%). The fecal samples were collected per rectum, pooled and filtered through a double layer of cheesecloth, diluted (1:6) in NaCl solution, homogenized, and added to each flask (5 mL) containing medium (79 mL) under CO_2 flow. The flasks were incubated at 39 °C for 72 h. During the incubation, gas pressure and volume were manually measured (14 times) using a pressure transducer (Cole and Parmer Instrument Co., Vernon Hills, IL, USA); the final gas production was related to incubated organic matter (OMCV, mL g^{-1}). The organic matter degradability (OMD, %) was determined by filtering flask content through #2 porosity glass crucibles and burning at 550 °C [19].

Fermentation liquor was collected from each flask after 72 h to determine pH and short-chain fatty acid (SCFA, mmol g^{-1}) production by gas chromatography (Thermo Fisher Scientific, Rodano, MI, Italy; model trace 1310) [24]. Branched-chain fatty acid proportion (BCFA) was also calculated as follows:

$$[(\text{iso-valerate} + \text{iso-butyrate})/\text{SCFA}] \quad (1)$$

2.6. Statistical Analysis

Agronomic (seed yield, thousand seed weight) and chemical (seed composition) in vitro fermentation parameters (OMCV, OMD, pH) and end-products (SCFA) were statistically analyzed by NESTED ANOVA (JMP®, Version 14 SW, SAS Institute Inc., Cary, NC, USA, 1989–2019) as follows:

$$y_{ijk} = \mu + acc_i + spec(acc)_{ij} + \varepsilon_{ijk} \quad (2)$$

where y_{ijk} is the observation k in level i of factor accession and level j of factor species; μ is the overall mean; acc_i is the effect of level i of factor accession; $spec(acc)_{ij}$ is the effect of level j of factor species within level i of factor accession; ε_{ijk} is the random error with mean 0 and variance σ^2.

When the main effects were significant, means were separated by the Tukey honest significance test at a 95% confidence level.

In vitro fermentation parameters and seed chemical composition, and the in vitro fermentation and fatty acid profile and peroxidation index were further assessed for significant correlations (JMP®, Version 14 SW, SAS Institute Inc., Cary, NC, USA, 1989–2019).

3. Results

3.1. Agronomic Traits

Seed yield was significantly different among amaranth accessions (Figure 1). The highest seed yield was registered in Pennsylvania (436.7 g m^{-2}) and the lowest in Taiwan (135.6 g m^{-2}). Benin did not differ from Pennsylvania and Arizona. This latter was similar to India, Iowa, and Goias. The remaining accessions, namely, Shaba, Delaware, Ohio, Beijing1, and Beijing2, did not differ from Goias. Among the four species, *A. cruentus* and *A. hypochondriacus* showed similar seed yields (on average, 322.1 g m^{-2}) that were greater than those of *A. hybridus* and *A. tricolor* (on average, 160.2 g m^{-2}).

Thousand seed weight (TSW) was significantly different among amaranth accessions (Figure 2). The highest TSW was found in Taiwan (0.90 g) and the lowest in Delaware and Goias (on average, 0.28 g). India and Iowa did not differ from Taiwan or Beijing1 and Beijing2. These two latter did not differ from Arizona. The remaining accessions, namely, Benin, Shaba, and Ohio, had significantly different TSW means. Among the four species, *A. tricolor* and *A. hypochondriacus* showed the highest TSW (on average, 0.81 g), followed by *A. cruentus* (0.56 g). The lowest TSW was observed in *A. hybridus* (0.29 g).

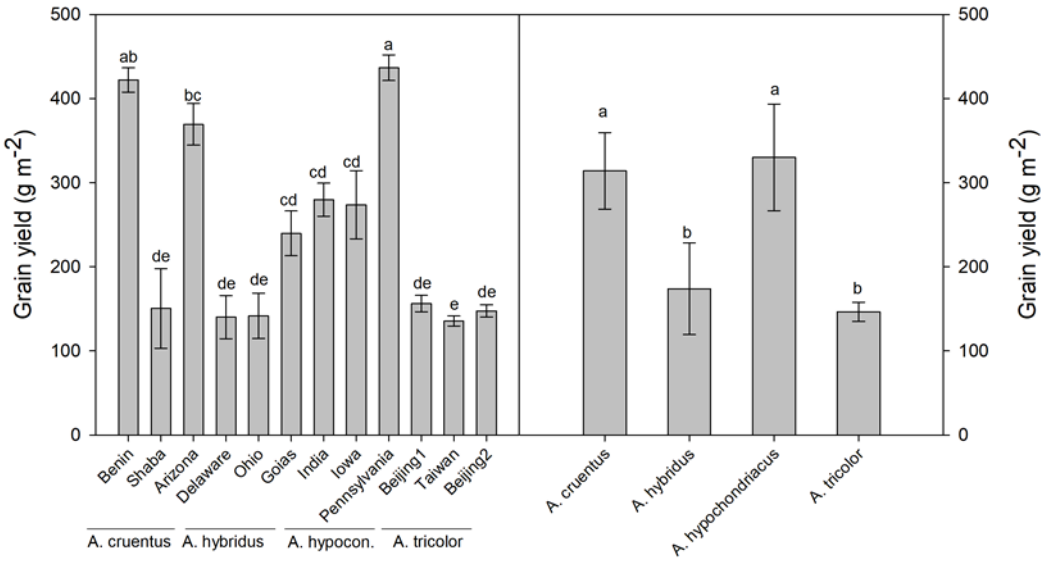

Figure 1. Seed yield (g m^{-2}) of the twelve amaranth accessions belonging to *A. cruentus*, *A. hybridus*, *A. hypochondriacus*, and *A. tricolor* species. Means ± standard errors followed by different letters indicate significant differences ($p \leq 0.05$).

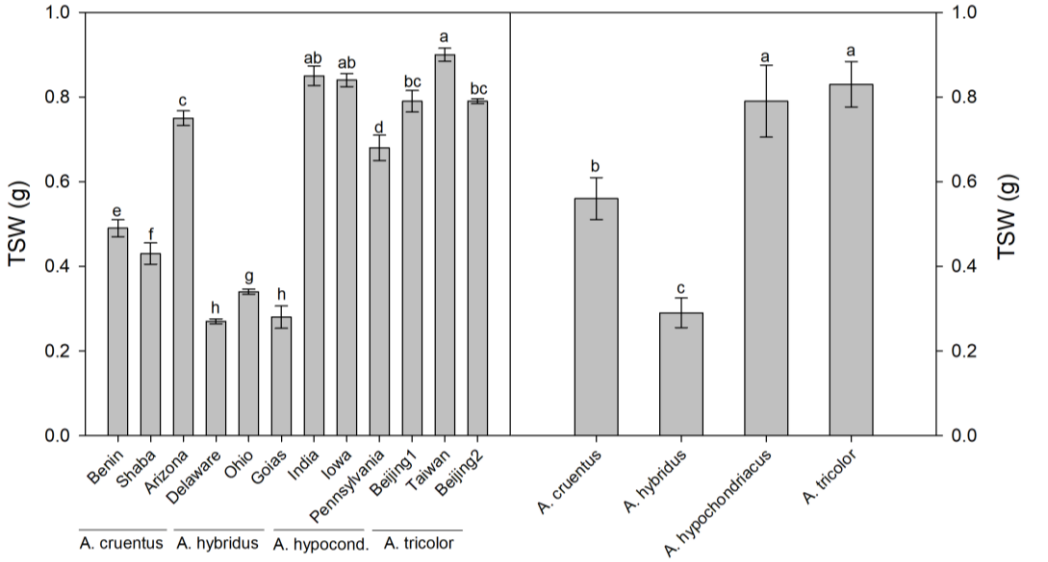

Figure 2. Thousand seed weight (TWS, g) of the twelve amaranth accessions belonging to *A. cruentus*, *A. hybridus*, *A. hypochondriacus*, and *A. tricolor* species. Means ± standard errors followed by different letters indicate significant differences ($p \leq 0.05$).

3.2. Proximate Chemical Composition

The chemical compositions of the four amaranth seed species and accessions are reported in Table 2. Considering species effect, significant differences ($p \leq 0.001$) were shown. *A. cruentus* had the highest values of CP and EE and the lowest SDF. *A. hypochondriacus* showed the highest values of CP, starch, and SDF and the lowest values of EE, ash, CF, and IDF. *A. hybridus* had the lowest values of CP and starch and the highest values of CF, TDF, and IDF, while *A. tricolor* showed the lowest value of ash, intermediate values for all the other parameters, and the highest value of TDF.

Table 2. Chemical composition (g 100 g^{-1}, as fed) of the twelve amaranth accessions belonging to *A. cruentus*, *A. hybridus*, *A. hypochondriacus*, and *A. tricolor* species.

Species		DM	Ash	CP	CF	EE	Starch	TDF	IDF	SDF
Species Effect										
A. cruentus		89.0 ^{C}	3.32 ^{B}	16.1 ^{A}	10.9 ^{B}	6.68 ^{A}	50.2 ^{C}	14.9 ^{B}	14.0 ^{C}	0.88 ^{D}
A.hybridus		89.0 ^{C}	3.22 ^{C}	14.4 ^{B}	16.2 ^{A}	5.91 ^{B}	47.6 ^{D}	19.3 ^{A}	18.0 ^{A}	1.26 ^{C}
A.hypochondriacus		89.7 ^{A}	3.04 ^{D}	16.0 ^{A}	4.93 ^{C}	5.56 ^{C}	51.5 ^{A}	14.5 ^{C}	12.5 ^{D}	2.03 ^{A}
A.tricolor		89.1 ^{B}	3.86 ^{A}	14.6 ^{B}	11.4 ^{B}	6.13 ^{B}	50.7 ^{B}	16.8 ^{A}	15.0 ^{B}	1.62 ^{B}
	Origin	**Accession Effect**								
A. cruentus	Benin	88.6 ^{E}	3.29 ^{CD}	16.1 ^{b}	11.7 ^{CD}	5.79 ^{CD}	49.4 ^{DE}	15.4 ^{DE}	14.5 ^{CD}	0.90 ^{EF}
	Shaba	88.9 ^{D}	3.23 ^{D}	16.3 ^{b}	10.5 ^{E}	5.68 ^{CD}	49.3 ^{DE}	15.5 ^{CD}	14.3 ^{C}	1.26 ^{CDE}
	Arizona	89.6 ^{B}	3.44 ^{BC}	16.0 ^{b}	10.5 ^{E}	6.25 ^{BCD}	51.8 ^{AB}	13.6 ^{FG}	13.2 ^{DE}	0.47 ^{F}
A.hybridus	Delaware	88.9 ^{CD}	3.20 ^{DE}	13.9 ^{c}	16.6 ^{A}	6.63 ^{AB}	48.2 ^{E}	19.5 ^{A}	18.2 ^{AB}	1.35 ^{CDE}
	Ohio	89.0 ^{CD}	3.17 ^{DE}	14.1 ^{c}	14.7 ^{B}	6.13 ^{BCD}	49.4 ^{D}	18.0 ^{B}	17.1 ^{B}	0.89 ^{EF}
	Goias	89.1 ^{CD}	3.29 ^{CD}	15.4 ^{bc}	17.2 ^{A}	7.27 ^{A}	45.2 ^{F}	20.2 ^{A}	18.7 ^{A}	1.55 ^{BCD}
A.hypochondriacus	India	89.7 ^{AB}	3.01 ^{EF}	16.4 ^{ab}	4.34 ^{F}	4.92 ^{E}	51.5 ^{B}	14.3 ^{EFG}	12.0 ^{E}	2.33 ^{A}
	Iowa	89.7 ^{AB}	3.17 ^{DE}	15.5 ^{bc}	5.25 ^{F}	5.64 ^{D}	51.2 ^{BC}	14.8 ^{DE}	13.0 ^{DE}	1.79 ^{ABC}
	Pennsylvania	89.9 ^{A}	2.93 ^{F}	15.9 ^{b}	5.18 ^{F}	6.14 ^{BCD}	51.7 ^{AB}	14.5 ^{DEF}	12.5 ^{E}	1.98 ^{AB}
A.tricolor	Beijing1	89.2 ^{C}	4.76 ^{A}	18.1 ^{A}	10.9 ^{DE}	5.79 ^{CD}	49.3 ^{DE}	13.3 ^{F}	12.3 ^{E}	1.04 ^{DEF}
	Taiwan	89.2 ^{C}	3.32 ^{BCD}	14.1 ^{C}	11.1 ^{CDE}	6.32 ^{BC}	52.8 ^{A}	14.4 ^{DEFG}	12.4 ^{E}	2.03 ^{AB}
	Beijing2	89.1 ^{CD}	3.49 ^{B}	15.0 ^{BC}	12.1 ^{C}	6.31 ^{BC}	50.1 ^{CD}	16.8 ^{BC}	15.0 ^{C}	1.79 ^{ABC}
RMSE		0.01	0.002	0.17	0.08	0.05	0.01	0.07	0.07	0.02

DM = dry matter; CP = crude protein; CF = crude fiber; EE = ether extract; TDF = total dietary fiber; IDF = insoluble dietary fiber; SDF = soluble dietary fiber. RMSE = mean square error. Along the column, lowercase and uppercase letters indicate $p \leq 0.05$ and 0.001, respectively.

Regarding accession effects, Beijing1 showed the highest values of CP and ash and the lowest value of IDF; Delaware showed the highest values of CF and TDF and the lowest value of CP; Goias showed the highest values of CF, EE, IDF, and TDF and the lowest value of starch; Taiwan showed the highest value of starch and the lowest value of CP and IDF. India, Iowa, and Pennsylvania had the lowest CF (on average, 4.9 g 100g^{-1}, as fed); India also showed the lowest values of EE and IDF, the highest value of SDF, and a high level of CP. The lowest value of SDF was found in the Arizona accession.

3.3. Fatty Acids, Fatty Acid Classes, and Peroxidation Index

Regarding the individual fatty acids in the four species (Table 3), significant differences ($p \leq 0.05$) were observed. In particular, *A. cruentus* showed the significantly highest levels of palmitic (C16:0) and oleic (C18:1n9) acids and the lowest levels of myristic (C14:0) and linoleic (C18:2n6) acids. *A. hybridus* showed the significantly highest levels of myristic and stearic (C18:0) acids and the lowest levels of oleic and alpha-linolenic (C18:3n3) acids; *A. hypochondriacus* showed the highest levels of alpha-linolenic acids and the lowest level of stearic acid. Finally, *A. tricolor* exhibited the highest level of linoleic acid and the lowest levels of saturated fatty acids as myristic, palmitic, and stearic acids.

Regarding the effect of the accessions on the fatty acids of nutritional interest (Table 3), the significantly highest levels were observed for palmitic acid in Shaba, oleic acid in Arizona, myristic and oleic acids in Delaware, linoleic acid in Ohio, and alpha-linolenic acid in India. The significantly lowest values were observed for myristic acid in all the accessions of *A. tricolor* (Beijing1, Taiwan, Beijing2), with an average value of 0.34 g 100g^{-1}, for palmitic and oleic acids in Beijing1. Oleic acid was the lowest in Delaware and Ohio; stearic acid was lowest in Taiwan and Beijing2; linoleic acid was lowest in Shaba, Arizona, and Goias; and alpha-linolenic acid was lowest in Delaware.

Table 3. Fatty acid profiles (g $100g^{-1}$) of the twelve amaranth accessions belonging to *A. cruentus*, *A. hybridus*, *A. hypochondriacus*, and *A. tricolor* species.

Species		C14:0	C16:0	C16:1	C17:0	C18:0	C18:1n9	C18:1n7	C18:2n6	C18:3n3	C20:0	C22:0	Others
							Species Effect						
A. cruentus		0.60 ^{c}	28.15 ^{a}	0.28 ^{ab}	0.60 ^{a}	6.52 ^{b}	29.38 ^{a}	1.42 ^{b}	29.38 ^{c}	0.38 ^{b}	1.02 ^{b}	0.46 ^{b}	1.82 ^{c}
A.hybridus		2.49 ^{a}	26.38 ^{c}	0.27 ^{ab}	0.50 ^{b}	8.10 ^{a}	25.25 ^{d}	1.40 ^{b}	32.77 ^{b}	0.31 ^{d}	0.96 ^{c}	0.40 ^{d}	1.19 ^{d}
A.hypochondriacus		0.72 ^{b}	27.41 ^{b}	0.32 ^{a}	0.43 ^{c}	5.89 ^{c}	27.15 ^{b}	1.43 ^{b}	32.83 ^{b}	0.54 ^{a}	0.90 ^{d}	0.44 ^{c}	1.96 ^{b}
A.tricolor		0.34 ^{d}	25.29 ^{d}	0.23 ^{b}	0.13 ^{d}	4.59 ^{d}	26.13 ^{c}	2.30 ^{a}	36.45 ^{a}	0.35 ^{c}	1.19 ^{a}	0.71 ^{a}	2.29 ^{a}
	Origin						**Accession Effect**						
A. cruentus	Benin	0.78 ^{d}	29.47 ^{b}	0.33 ^{ab}	0.65 ^{b}	7.13 ^{d}	28.58 ^{b}	1.43 ^{b}	28.04 ^{f}	0.36 ^{efg}	1.06 ^{cd}	0.48 ^{e}	1.72 ^{f}
	Shaba	0.64 ^{e}	30.79 ^{a}	0.28 ^{b}	0.70 ^{a}	7.44 ^{c}	28.53 ^{b}	1.41 ^{b}	26.08 ^{g}	0.33 ^{gh}	1.25 ^{b}	0.56 ^{d}	2.03 ^{de}
	Arizona	0.40 ^{g}	24.20 ^{g}	0.23 ^{b}	0.44 ^{de}	5.00 ^{h}	31.02 ^{a}	1.42 ^{b}	34.03 ^{d}	0.45 ^{c}	0.76 ^{f}	0.35 ^{h}	1.72 ^{f}
A.hybridus	Delaware	3.81 ^{a}	29.02 ^{c}	0.31 ^{ab}	0.53 ^{c}	10.35 ^{a}	24.17 ^{f}	1.44 ^{b}	27.68 ^{f}	0.20 ^{i}	0.99 ^{d}	0.41 ^{fg}	1.12 ^{h}
	Ohio	0.57 ^{f}	20.72 ^{i}	0.18 ^{b}	0.46 ^{d}	5.44 ^{g}	24.00 ^{f}	1.27 ^{c}	44.21 ^{a}	0.41 ^{cd}	0.85 ^{ef}	0.36 ^{h}	1.57 ^{g}
	Goias	3.10 ^{b}	29.42 ^{b}	0.31 ^{ab}	0.51 ^{c}	8.50 ^{b}	27.58 ^{c}	1.51 ^{b}	26.42 ^{g}	0.33 ^{fgh}	1.05 ^{cd}	0.43 ^{f}	0.88 ^{i}
A.hypochondriacus	India	0.75 ^{d}	27.04 ^{d}	0.24 ^{b}	0.42 ^{e}	5.90 ^{f}	26.45 ^{e}	1.43 ^{b}	33.96 ^{d}	0.63 ^{a}	0.85 ^{e}	0.42 ^{fg}	1.96 ^{e}
	Iowa	0.59 ^{f}	26.23 ^{e}	0.29 ^{b}	0.36 ^{f}	5.38 ^{g}	28.43 ^{b}	1.43 ^{b}	33.84 ^{d}	0.54 ^{b}	0.82 ^{ef}	0.40 ^{g}	1.71 ^{f}
	Pennsylvania	0.83 ^{c}	28.96 ^{c}	0.45 ^{a}	0.52 ^{c}	6.39 ^{e}	26.58 ^{de}	1.44 ^{b}	30.70 ^{e}	0.44 ^{c}	1.03 ^{d}	0.49 ^{e}	2.21 ^{c}
A.tricolor	Beijing1	0.34 ^{h}	23.78 ^{h}	0.25 ^{b}	0.12 ^{h}	5.13 ^{h}	23.96 ^{f}	2.24 ^{a}	39.14 ^{b}	0.38 ^{de}	1.36 ^{a}	0.86 ^{a}	2.47 ^{a}
	Taiwan	0.35 ^{h}	25.09 ^{f}	0.22 ^{b}	0.13 ^{gh}	4.25 ^{i}	27.14 ^{cd}	2.34 ^{a}	36.02 ^{c}	0.38 ^{def}	1.30 ^{c}	0.62 ^{c}	2.32 ^{b}
	Beijing2	0.34 ^{h}	27.02 ^{d}	0.21 ^{b}	0.15 ^{g}	4.40 ^{i}	27.30 ^{c}	2.31 ^{a}	34.20 ^{d}	0.31 ^{h}	1.08 ^{cd}	0.66 ^{b}	2.07 ^{d}
RMSE		0.014	0.132	0.051	0.009	0.051	0.231	0.038	0.204	0.015	0.030	0.011	0.011

The concentration of fatty acids was expressed as g $100g^{-1}$, considering 100 g as the sum of the areas of all identified FAMEs. C14:0 = myristic acid; C16:0 = palmitic acid; C16:1 = palmitoleic acid; C17:0 = heptadecanoic acid; C18:0 = stearic acid; C18:1n9 = oleic acid; C18:1n7 = cis-vaccenic acid; C18:2n6 = linoleic acid; C18:3n3 = α-linolenic acid; C20:0 = arachidic acid; C22:0 = behenic acid. RMSE: root mean square error; along the column, letters indicate $p \leq 0.05$. Regarding the fatty acid classes (Table 4) in the four species, *A. hybridus* had the significantly highest values of saturated fatty acids (SFA) and the SFA/UFA (unsaturated fatty acids) ratio. *A. cruentus* showed the highest monounsaturated fatty acids (MUFA), and *A. tricolor* the highest polyunsaturated fatty acids (PUFA) and the highest levels of the PUFA of the n6-series (n6-PUFA) and of the peroxidation index (PI). *A. hypochondriacus* showed the highest PUFA levels of the n3-series (n3-PUFA). The significantly lowest levels of fatty acid classes, ratios, and quality indexes were observed in *A. tricolor* (SFA and SFA/UFA ratio), in *A. hybridus* (MUFA and n3-PUFA), and in *A. cruentus* (PUFA, n6-PUFA, and PI).

Table 4. Fatty acid classes (g $100g^{-1}$), SFA/UFA ratios, and peroxidation indexes of the twelve amaranth accessions belonging to *A. cruentus*, *A. hybridus*, *A. hypochondriacus*, and *A. tricolor* species.

Species		SFA	MUFA	PUFA	SFA/UFA	n3-PUFA	n6-PUFA	PI
				Species Effect				
A. cruentus		37.35 ^{b}	31.08 ^{a}	29.76 ^{c}	0.62 ^{b}	0.38 ^{b}	29.38 ^{c}	30.13 ^{d}
A.hybridus		38.82 ^{a}	26.92 ^{c}	33.08 ^{b}	0.67 ^{a}	0.31 ^{d}	32.77 ^{b}	33.39 ^{c}
A.hypochondriacus		35.78 ^{c}	28.90 ^{b}	33.36 ^{b}	0.58 ^{c}	0.54 ^{a}	32.83 ^{b}	33.89 ^{b}
A.tricolor		32.25 ^{d}	28.65 ^{b}	36.80 ^{a}	0.49 ^{d}	0.35 ^{c}	36.45 ^{a}	37.16 ^{a}
	Origin				**Accession Effect**			
A. cruentus	Benin	39.56 ^{d}	30.35 ^{b}	28.39 ^{f}	0.68 ^{d}	0.36 ^{efg}	28.04 ^{f}	28.74 ^{f}
	Shaba	41.37 ^{c}	30.21 ^{b}	26.40 ^{g}	0.73 ^{c}	0.33 ^{gh}	26.08 ^{g}	26.73 ^{g}
	Arizona	31.14 ^{h}	32.67 ^{a}	34.48 ^{d}	0.47 ^{i}	0.45 ^{c}	34.03 ^{d}	34.93 ^{d}
A.hybridus	Delaware	45.10 ^{a}	25.92 ^{ef}	27.88 ^{f}	0.84 ^{a}	0.20 ^{i}	27.68 ^{f}	28.05 ^{f}
	Ohio	28.39 ^{i}	25.44 ^{f}	44.62 ^{a}	0.41 ^{j}	0.41 ^{cd}	44.21 ^{a}	45.02 ^{a}
	Goias	42.99 ^{b}	29.40 ^{c}	26.75 ^{g}	0.77 ^{b}	0.33 ^{fgh}	26.42 ^{g}	27.07 ^{g}
A.hypochondriacus	India	35.37 ^{f}	28.11 ^{d}	34.58 ^{d}	0.57 ^{f}	0.63 ^{a}	33.96 ^{d}	35.20 ^{d}
	Iowa	33.78 ^{g}	30.15 ^{bc}	34.38 ^{d}	0.52 ^{g}	0.54 ^{b}	33.84 ^{d}	34.91 ^{d}
	Pennsylvania	38.21 ^{e}	28.46 ^{d}	31.13 ^{e}	0.64 ^{e}	0.44 ^{c}	30.70 ^{e}	31.57 ^{e}
A.tricolor	Beijing1	31.56 ^{h}	26.44 ^{e}	39.52 ^{b}	0.48 ^{hi}	0.38 ^{de}	39.14 ^{b}	39.91 ^{b}
	Taiwan	31.57 ^{h}	29.70 ^{bc}	36.40 ^{c}	0.48 ^{h}	0.38 ^{def}	36.02 ^{c}	36.78 ^{c}
	Beijing2	33.63 ^{g}	29.82 ^{bc}	34.50 ^{d}	0.53 ^{g}	0.31 ^{h}	34.20 ^{d}	34.80 ^{d}
RMSE		0.161	0.265	0.216	0.005	0.015	0.204	0.227

The concentration of fatty acids was expressed as g $100g^{-1}$, considering 100 g as the sum of the areas of all identified FAMEs. SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; n3 = n3-polyunsaturated fatty acids; n6 = n6-polyunsaturated fatty acids; SFA/UFA = saturated/unsaturated fatty acid ratio; PI = peroxidation index; RMSE: root mean square error; along the column, letters indicate $p \leq 0.05$.

Among the accessions (Table 4), Shaba showed the lowest values of PUFA and PI; Arizona showed the highest MUFA; Delaware showed the highest SFA and SFA/UFA ratio; Ohio showed the highest PUFA, n6-PUFA, and PI and the lowest SFA, MUFA, and SFA/UFA ratio; India showed the highest n3-PUFA; and Beijing2 showed the lowest n3-PUFA.

3.4. In Vitro Fermentation Characteristics, Kinetics, and End-Products

In Table 5, the in vitro parameters results are described. *A. hypochondriacus* showed the highest percentage of OMD while *A. hybridus* showed the lowest (<50%). In addition, *A. hypochondriacus* registered the highest volume of gas produced. *A. hybridus* and *A. tricolor* reported the lowest gas production. Among the accessions, India exhibited the highest OMD, while Delaware had the lowest. The latter also showed the lowest level of OMCV. In Shaba, the highest gas production was recorded.

In Table 6, in vitro end-products are reported. *A. hypochondriacus* and *A. hybridus* showed the highest and lowest amount of SCFA, respectively. *A. hybridus* and *A. tricolor* reported the greatest percentages of BCFA, while *A. hypochondriacus* had the lowest. Furthermore, *A. hybridus* and *A. tricolor* exhibited the highest levels of acetate, iso-butyrate, iso-valerate, and valerate. *A. cruentus* showed the lowest percentage of propionate, while *A. hypochondriacus* had the highest. These two species produced the greatest percentages of butyrate. *A. hypochondriacus* produced the lowest amounts iso-butyrate, iso-valerate, and valerate. India, Iowa, and Pennsylvania were the accessions with the highest amounts of SCFA. Delaware, Goias, Shaba, and Beijing1 reported the lowest levels of SCFA. *A. hybridus* accessions (Delaware, Ohio, Goias) and *A. tricolor* accessions (Beijing1, Taiwan, and Beijing2) showed the highest levels of BCFA. On the contrary, *A. hypochondriacus* accessions resulted in the lowest level of BCFA. Beijing2 and Ohio showed the highest percentages of acetate. Iowa showed the lowest and highest levels of acetate and propionate, respectively. Shaba had the greatest amount of butyrate.

Table 5. In vitro fermentation characteristics of the twelve amaranth accessions belonging to *A. cruentus*, *A. hybridus*, *A. hypochondriacus*, and *A. tricolor* species.

Species		OMD (%)	OMCV (mL g^{-1})
		Species Effect	
A. cruentus		73.1 ^{B}	178 ^{B}
A. hybridus		46.8 ^{D}	107 ^{C}
A. hypochondriacus		77.6 ^{A}	182 ^{A}
A. tricolor		48.1 ^{C}	109 ^{C}
	Origin	**Accession Effect**	
A. cruentus	Benin	65.7 ^{D}	152 ^{D}
	Shaba	78.1 ^{AB}	193 ^{A}
	Arizona	75.3 ^{C}	190 ^{AB}
A. hybridus	Delaware	38.9 ^{I}	84.4 ^{H}
	Ohio	55.1 ^{E}	130 ^{E}
	Goias	46.4 ^{G}	106 ^{G}
A. hypochondriacus	India	80.0 ^{A}	185 ^{B}
	Iowa	76.3 ^{BC}	174 ^{C}
	Pennsylvania	76.3 ^{BC}	186 ^{AB}
A. tricolor	Beijing1	42.7 ^{H}	104 ^{G}
	Taiwan	49.9 ^{F}	115 ^{F}
	Beijing2	51.6 ^{F}	109 ^{G}
RMSE		0.87	6.14

OMD = organic matter degradability; OMCV = cumulative volume of gas related to incubated organic matter; RMSE = root mean square error; along the column, uppercase letters indicate $p < 0.01$.

Table 6. In vitro fermentation end-products of the twelve amaranth accessions belonging to *A. cruentus*, *A. hybridus*, *A. hypochondriacus*, and *A. tricolor* species.

Species		SCFA	BCFA	Ace	Prop	Iso-But	But	Iso-Val	Val
		mmol g^{-1}		% SCFA					
		Species effect							
A. cruentus		37.2 ^{C}	5.44 ^{B}	49.8 ^{B}	17.3 ^{D}	1.98 ^{B}	22.8 ^{A}	3.50 ^{B}	3.40 ^{B}
A. hybridus		31.6 ^{D}	6.78 ^{A}	52.3 ^{A}	22.4 ^{B}	2.48 ^{A}	15.1 ^{B}	4.24 ^{A}	4.07 ^{A}
A. hypochondriacus		52.6 ^{A}	4.68 ^{C}	49.0 ^{B}	24.4 ^{A}	1.64 ^{C}	20.7 ^{A}	3.17 ^{C}	2.76 ^{C}
A. tricolor		40.3 ^{B}	6.88 ^{A}	52.2 ^{A}	21.0 ^{C}	2.53 ^{A}	16.5 ^{B}	4.35 ^{A}	4.07 ^{A}
	Origin	**Accession effect**							
A. cruentus	Benin	38.5 ^{DE}	5.78 ^{BC}	52.3 ^{ABCD}	17.0 ^{FG}	2.00 ^{BC}	21.7 ^{ABC}	3.77 ^{BCD}	4.06 ^{A}
	Shaba	33.0 ^{EF}	5.46 ^{C}	49.1 ^{DEF}	16.8 ^{G}	1.99 ^{BC}	25.7 ^{A}	3.58 ^{CD}	3.46 ^{AB}
	Arizona	40.0 ^{CD}	5.09 ^{CD}	48.0 ^{EF}	18.0 ^{EFG}	1.94 ^{CD}	21.0 ^{ABCD}	3.15 ^{EF}	2.66 ^{B}
A. hybridus	Delaware	28.7 ^{F}	6.97 ^{A}	51.9 ^{ABCDE}	21.4 ^{CD}	2.60 ^{A}	14.7 ^{E}	4.37 ^{A}	4.13 ^{A}
	Ohio	36.6 ^{DE}	6.62 ^{AB}	53.1 ^{AB}	21.1 ^{CDE}	2.24 ^{B}	16.6 ^{CDE}	4.21 ^{ABC}	4.00 ^{A}
	Goias	29.3 ^{F}	6.74 ^{A}	51.8 ^{ABCDE}	24.7 ^{B}	2.60 ^{A}	14.1 ^{E}	4.14 ^{AB}	4.07 ^{A}
A. hypochondriacus	India	55.0 ^{A}	4.94 ^{D}	50.8 ^{BCDE}	20.6 ^{DEF}	1.84 ^{CD}	22.1 ^{ABC}	3.24 ^{DEF}	2.80 ^{B}
	Iowa	51.7 ^{A}	4.79 ^{D}	47.8 ^{F}	28.0 ^{A}	1.45 ^{E}	16.7 ^{CDE}	3.37 ^{DE}	2.84 ^{B}
	Pennsylvania	51.1 ^{AB}	4.30 ^{E}	48.3 ^{DEF}	24.5 ^{B}	1.64 ^{DE}	23.2 ^{AB}	2.90 ^{F}	2.64 ^{B}
A. tricolor	Beijing1	35.4 ^{DEF}	6.67 ^{A}	52.5 ^{ABC}	19.6 ^{DEFG}	2.50 ^{A}	17.8 ^{BCDE}	4.17 ^{AB}	4.32 ^{A}
	Taiwan	45.0 ^{BC}	7.04 ^{A}	50.1 ^{CDEF}	23.9 ^{BC}	2.57 ^{A}	14.9 ^{E}	4.46 ^{A}	4.03 ^{A}
	Beijing2	40.5 ^{CD}	6.94 ^{A}	53.9 ^{A}	19.6 ^{DEFG}	2.51 ^{A}	16.9 ^{CDE}	4.43 ^{A}	3.97 ^{A}
RMSE		6.40	0.03	1.08	1.11	0.01	5.15	0.03	0.10

Ace = acetate; Prop = propionate; Iso-But = iso-butyrate; But = butyrate; Iso-Val = iso-valerate; Val = valerate; SCFA = short-chain fatty acids; BCFA = branched-chain fatty acids (Iso-But + Iso-Val)/SCFA × 100; RMSE = root mean square error; along the column, uppercase letters indicate $p < 0.001$.

3.5. Correlation

The correlation between in vitro and chemical composition parameters is described in Table 7. The CP content showed a positive correlation ($p < 0.01$) with OMD, OMCV,

and butyrate, but was negatively correlated ($p < 0.05$) with BCFA, iso-butyrate, and iso-valerate. On the contrary, CF and EE were negatively correlated ($p < 0.01$, 0.05, respectively) with OMD, OMCV, and SCFA. However, both parameters showed positive correlations ($p < 0.01$ and 0.05, respectively) with BCFA, acetate, iso-butyrate, iso-valerate, and valerate. Similarly, starch content was positively correlated ($p < 0.01$) with OMD, OMCV, and SCFA ($p < 0.05$). On the contrary, starch was negatively correlated ($p < 0.01$) with BCFA, iso-butyrate, iso-valerate, valerate, and acetate ($p < 0.05$). Total dietary fiber, insoluble dietary fiber, and total dietary fiber and ash contents were negatively ($p < 0.05$) correlated with OMD, OMCV, SCFA, and butyrate and positively ($p < 0.01$) with BCFA, acetate, iso-butyrate, iso-valerate, and valerate. The SDF reported a positive ($p < 0.05$) correlation with SCFA and a negative correlation ($p < 0.05$) with valerate.

Table 7. Correlation between in vitro parameters and chemical composition.

	OMD	OMCV	SCFA	BCFA	Ace	Prop	Iso-But	But	Iso-Val	Val
CP	0.6642 **	0.7342 ***	0.0765 NS	−0.6422 **	−0.4182 NS	−0.3337 NS	−0.5608 *	0.7671 ***	−0.6596 **	−0.3179 NS
CF	−0.7754 ***	−0.7317 ***	−0.6690 ***	0.8604 ***	0.6691 ***	−0.3071 NS	0.8305 ***	−0.4206 NS	0.8321 ***	0.8399 ***
EE	−0.5640 **	−0.4951 *	−0.4083 *	0.4624 *	0.4130 *	−0.1011 NS	0.4913 *	−0.3145 NS	0.4131 *	0.4662 *
Starch	0.6964 ***	0.6613 ***	0.4737 *	−0.6904 ***	−0.4565 *	0.1718 NS	−0.6350 ***	0.3589 NS	−0.6916 ***	−0.7044 ***
IDF	−0.8577 ***	−0.8389 ***	−0.5574 *	0.8937 ***	0.6036 **	−0.1498 NS	0.8407 ***	−0.5747 *	0.8802 ***	0.7927 ***
SDF	0.1493 NS	0.0743 NS	0.5340 *	−0.3098 NS	−0.2836 NS	0.2644 NS	−0.3234 NS	0.0754 NS	−0.2809 NS	−0.4665 *
TDF	−0.8719 ***	−0.8707 ***	−0.4585 *	0.8639 ***	0.5666 *	−0.0609 NS	0.8089 ***	−0.6094 **	0.8536 ***	0.7144 ***
Ash	−0.5372 *	−0.5201 *	−0.6731 **	0.5372 *	0.3638 NS	0.2626 NS	0.6056 **	−0.5910 **	0.4488 *	0.4853 *

CP = crude protein; CF = crude fiber; EE = ether extract; IDF = insoluble dietary fiber; SDF = soluble dietary fiber; TDF = total dietary fiber; OMD = organic matter degradability; OMCV = cumulative volume of gas related to incubated organic matter; Ace = acetate; Prop = propionate; Iso-But = iso-butyrate; Iso-Val = iso-valerate; SCFA = short-chain fatty acids; BCFA = branched-chain fatty acids (Iso-But + Iso-Val)/SCFA × 100. *, **, ***, and NS indicate $p < 0.001$, 0.01, 0.05, and not significant, respectively.

Table 8 provides the correlation between in vitro fermentation parameters, fatty acid classes, and quality index. Myristic acid was positively correlated ($p < 0.05$) with BCFA and iso-valerate. Palmitic acid was positively correlated ($p < 0.05$) with OMD and SCFA, while palmitoleic acid was positively correlated ($p < 0.05$) with SCFA. Differently, oleic acid was highly ($p < 0.001$) and positively correlated with OMD, OMCV, and butyrate. On the contrary, the same fatty acid was negatively correlated with BCFA, acetate, iso-butyrate, and iso-valerate. Linoleic acid was negatively correlated ($p < 0.05$) with OMD. Alpha-linolenic acid was positively correlated with OMD, OMCV, SCFA, and butyrate and negatively with BCFA, acetate, iso-butyrate, iso-valerate, and valerate.

Regarding the classes, MUFA were positively correlated with OMD, OMCV, and butyrate, while they were negatively correlated with iso-valerate. The polyunsaturated fatty acids of the n3 series were positively correlated to OMD, OMCV, SCFA, and butyrate. These fatty acids were negatively correlated with BCFA, acetate, iso-butyrate, iso-valerate, and valerate. The PUFA-n6 series were negatively correlated with OMD.

Table 8. Correlation between in vitro parameters, fatty acid classes, and quality index.

	OMD	OMCV	SCFA	BCFA	Ace	Prop	Iso-But	But	Iso-Val	Val
C14:0	−0.227 NS	−0.294 NS	0.212 NS	0.428 *	0.226 NS	0.053 NS	0.320 NS	−0.282 NS	0.483 *	0.161 NS
C16:0	0.406 *	0.351 NS	0.438 *	−0.273 NS	−0.027 NS	0.058 NS	−0.329 NS	0.071 NS	−0.213 NS	−0.294 NS
C16:1	0.3011 NS	0.2067 NS	0.4265 *	−0.179 NS	−0.172 NS	0.267 NS	−0.368 NS	−0.043 NS	−0.025 NS	−0.249 NS
C17:0	0.4715 *	0.4778 *	0.3964 NS	−0.275 NS	−0.160 NS	−0.301 NS	−0.370 NS	0.413 *	−0.187 NS	−0.179 NS
C18:0	−0.011 NS	−0.068 NS	0.280 NS	0.218 NS	0.202 NS	−0.090 NS	0.099 NS	−0.089 NS	0.295 NS	0.025 NS
C18:1n9	0.680 ***	0.709 ***	0.131 NS	−0.448 *	−0.448 *	−0.367 NS	−0.406 *	0.659 ***	−0.454 *	−0.172 NS
C18:1n7	−0.521 **	−0.518 **	−0.555 **	0.347 NS	0.338 NS	0.274 NS	0.445 *	−0.524 **	0.252 NS	0.314 NS
C18:2n6	−0.409 *	−0.359 NS	−0.365 NS	0.171 NS	0.064 NS	0.074 NS	0.238 NS	−0.160 NS	0.111 NS	0.191 NS
C18:3n3	0.532 **	0.550 **	0.469 *	−0.665 ***	−0.687 ***	0.154 NS	−0.634 ***	0.498 *	−0.648 ***	−0.687 ***
C20:0	−0.432 *	−0.435 *	−0.412 *	0.348 NS	0.372 NS	0.258 NS	0.363 NS	−0.524 **	0.316 NS	0.268 NS
C22:0	−0.461 *	−0.457 *	−0.500 *	0.266 NS	0.333 NS	0.271 NS	0.321 NS	−0.485 *	0.2078 NS	0.201 NS
SFA	0.163 NS	0.099 NS	0.371 NS	0.024 NS	0.119 NS	0.019 NS	−0.072 NS	−0.067 NS	0.095 NS	−0.112 NS
MUFA	0.601 ***	0.627 ***	0.044 NS	−0.395 NS	−0.396 NS	−0.308 NS	−0.342 NS	0.564 **	−0.411 *	−0.124 NS
PUFA	−0.396 NS	−0.345 NS	−0.353 NS	0.157 NS	0.050 NS	0.076 NS	0.223 NS	−0.148 NS	0.097 NS	0.176 NS
SFA/UFA	0.128 NS	0.066 NS	0.336 NS	0.061 NS	0.147 NS	0.010 NS	−0.036 NS	−0.089 NS	0.130 NS	−0.079 NS
n3-PUFA	0.532 **	0.550 **	0.469 *	−0.665 ***	−0.687 ***	0.154 NS	−0.634 ***	0.498 *	−0.648 ***	−0.687 ***
n6-PUFA	−0.409 *	−0.359 NS	−0.365 NS	0.171 NS	0.064 NS	0.074 NS	0.2378 NS	−0.160 NS	0.111 NS	0.191 NS
PI	−0.382 NS	−0.332 NS	−0.341 NS	0.142 NS	0.035 NS	0.079 NS	0.209 NS	−0.13 NS	0.084 NS	0.161 NS

C14:0 = myristic acid; C16:0 = palmitic acid; C16:1 = palmitoleic acid; C17:0 = heptadecanoic acid; C18:0 = stearic acid; C18:1n9 = oleic acid; C18:1n7 = vaccenic acid; C18:2n6 = linoleic acid (LA); C18:3n3 = α-linoleic acid (ALA); C20:0 = arachidic acid; C22:0 = behenic acid; OMD = organic matter degradability; OMCV = cumulative volume of gas related to incubated organic matter; Ace = acetate; Prop = propionate; Iso-But = iso-butyrate; Iso-Val = iso-valerate; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; n3 = n3 polyunsaturated fatty acids; n6 = n6 polyunsaturated fatty acids; PI = peroxidation index. *, **, ***, and NS indicate $p < 0.001$, 0.01, 0.05, and not significant, respectively.

4. Discussion

Across the average of investigated accessions, *A. tricolor* and *A. hypochondriacus* showed the significantly highest thousand seed weight (TSW), with an accession range of 0.79–0.90 g for *A. tricolor* and 0.68–0.85 g for *A. hypochondriacus.* The present TSW range of *A. hypochondriacus* is slightly higher than that found by Pospišil et al. [25], who reported 0.69–0.73 g in *A. hypochondriacus* across the average of nitrogen fertilization levels in a three-year field trial in Zagreb, Croatia. The *A. tricolor* TSW range was also wider as com-

pared with 10 genotypes of *A. tricolor* (0.73–0.85 g) grown in Raipur, India [26]. Given the high polymorphism of accessions within the same amaranth species, differences might be ascribed to the genotype effects. However, growing conditions cannot be ruled out as demonstrated by Pospišil et al. [25], who pointed out a lower TSW of *A. hypochondriacus* under drier than more favorable years. In this study, *A. cruentus* showed lower TSW than the previous two species (0.43–0.75 g), and this somehow contrasts with results found in the literature of similar TSW between *A. hypochondriacus* and *A. cruentus* [25,27]. Nonetheless, our TSW with *A. cruentus* is within the range reported by Rivelli et al. [28] in a comparison of five different accessions of *A. cruentus* tested in South Italy. The *A. hybridus* accessions showed the lowest TWS (0.27–0.34 g) and it is similar to the lowest range found by Rivelli et al. [28]. However, this contrasts with Parveen et al. [29], who reported a TSW of 0.55 g averaging eight *A. hybridus* genotypes.

Seed yield was similar between *A. cruentus* and *A. hypochondriacus*, which agrees with the findings of Pospišil et al. [25], who demonstrated no genotypic differences between *A. cruentus* and *A. hypochondriacus* in two out of three growing seasons. However, we found a larger accession influence on grain yield for *A. cruentus* than for *A. hypochondriacus*, as the coefficient of variation of accessions was 46% and 28%, respectively. *A. hybridus* and *A. tricolor* were both less productive, and the coefficient of variation of accessions was quite narrow for *A. tricolor* (7.1%) as compared to *A. hybridus* (32.8%). Grain yields of the best accessions (Pennsylvania, Benin, Shaba) were well comparable or even higher than most cereals grown in semiarid Mediterranean environments, such as durum wheat [30,31] and oat [32].

In contrast to Pospišil et al. [25], who found an increase in seed yield associated with the increase in TSW in a dry growing season, our findings did not show a significant correlation between seed yield and TSW ($p = 0.667$, data not shown). However, the same author pointed out no correlation between seed yield and TSW with more favorable growing conditions. It is worth mentioning that in the present study, the air temperature was typical of the experimental area and rainfall was well distributed [11]; this suggests that traits other than TSW, such as the seed number per panicle or the number of panicles per plant, might have influenced seed yield of investigated accessions.

The organic matter degradability (OMD) of tested *Amaranthus* spp. was quite low for all samples. In particular, for *A. tricolor* and *A. hybridus* species, the OMD was less than 50%. These results could be ascribed to the presence of insoluble dietary fiber in the tested amaranth, as suggested by the significant negative correlations (Table 7) between the insoluble fiber and OMD and OMCV values (−0.8577 and −0.8389, respectively).

On the other hand, insoluble fiber helps to maintain normal gut function but might decrease feed intake and nutrient digestibility [33], increasing the rate of gut passage [34]. Moreover, Acosta et al. [35] tested the addition of distillers dried grains in pig diet, observing that starch digestibility can be affected by insoluble fiber level. Despite the lack of an enzymatic digestion test in this study, the starch content positively affected the fermentation parameters (0.6964 and 0.6613 for OMD and OMCV, respectively).

Starchy feeds as a source of energy and raw cereal grains, along with legume grains and potato starch, constitute the main dietary starch source in pig rations [36]. The concentration of SCFA was quite low for all tested samples; the presence of insoluble dietary fiber could affect the fermentation pathway, as demonstrated by the limited production of SCFA of *A. hybridus* and some accessions of *A. tricolor* (Beijing1 and Beijing2). *A. hypochondriacus* showed higher production of SCFA and butyrate probably due to the higher proportion of soluble dietary fiber, which is readily fermentable [37]. In this regard, the correlations between chemical composition and fermentation parameters demonstrated a positive correlation between starch, SDF vs. SCFA. As suggested by Weaver et al. [38], SDF produced more SCFA compared to IDF. Furthermore, starch feeds that bypass digestion in the stomach and enzymatic hydrolysis in the small intestine are fermented in the large intestine, producing SCFA [39]. Furthermore, the content of crude protein positively affected the fermentation process, particularly butyrate production (0.7671). The high proportion of

butyrate could be useful for the colonic epithelium as a main energy source for cell growth and differentiation [40], suggesting a potential pre-biotic role of *A. hypochondriacus*.

In swine nutrition, it is well known that the dietary FA composition and the molecular structures (chain length and number of double bonds) influence digestion, absorption, and metabolism, and the bioactivity of the FA [41]. The manipulation of lipids in pig diet, especially in the chain length of dietary FA, may be a strategic tool to improve animal performance [41], as explained by the complexity of digestion and absorption of these molecules. The addition of lipids to diets can, in turn, enhance protein digestibility by slowing the passage rate in the intestine by lipids, which contrasts with the effect of fiber [42]. However, still, little focus has been devoted to the impact of lipids, in terms of fatty acids, particularly on gut health and the development of early nutrition of pigs [43].

In general, pigs digest unsaturated dietary lipids more than saturated lipids [44]. Hence, lipase activity and lipid digestion can be positively influenced by unsaturated long-chain fatty acids (LCFA) and negatively by saturated LCFA [41]. Moreover, among unsaturated LCFA, those of the n3 series are believed to affect the gut microbiota mainly through regulation of the type and number of bacteria in the gut, and regulation of SCFA concentrations [45]. This could explain the results obtained in this study where oleic and alpha-linolenic acids as well as total MUFA and n3-PUFA showed positive correlations with OMD (0.680, 0.532, 0.601, 0.532, respectively) and OMCV (0.709, 0.550, 0.627, 0.550, respectively). Conversely, among unsaturated LCFA, linoleic acid and n6-PUFA showed negative correlations with OMD (−0.409) or were not significantly correlated with OMCV. To further confirm these observations, a positive correlation for alpha-linolenic acid and n3-PUFA with SCFA (0.469) and a non-significant correlation between n6-PUFA and SCFA was found. Among the varieties, *A. hypochondriacus,* with the highest content of alpha-linolenic acid and n3-PUFA (0.54 g $100g^{-1}$), presented the highest OMD (77.6%), OMCV (182 mL g^{-1}), and SCFA production (52.6 mmol g^{-1}) and the highest levels of propionate and butyrate (24.2% and 20.7% SCFA, respectively). From a nutritional point of view, it is noteworthy that the metabolites produced by the gut microbiota significantly influence the host's metabolism and health [46]. The proportion of SCFA, produced by bacterial fermentation in the gut, exerts several effects on the host's metabolism and immune system [47]. SCFA, produced via microbial fermentation of non-digestible carbohydrates and digestible starch in the hindgut, contribute with an energy supply for the host and the colonocytes [48] and have antimicrobial properties limiting the risk of infectious diseases in the gut [41].

5. Conclusions

Among the studied species, *A. hypochondriacus* emerged not only for its desirable nutritive value, agronomic traits, and suitability to Mediterranean growing conditions, but also for potential beneficial effects. Although the thousand seed weight was in the lowest range group in Pennsylvania (*A. hypochondriacus*) and Benin (*A. cruentus*), these accessions outyielded the others, suggesting greater adaptability to the Mediterranean growing conditions. With the highest CP, starch, and SDF content and a good proportion of fatty acids, favorable in vitro fermentation characteristics with the highest SCFA and butyric production, together with the lowest CF and IDF content, *A. hypochondriacus* exhibits not only a suitable nutritive value but also a potentially positive effect on large intestine status.

Nonetheless, further field trials with a larger set of accessions from the available amaranth germplasm in multiple sites will help understand the environmental effect on genotypes and to draw clear conclusions on the effectiveness of this crop. Moreover, additional information on amaranth's nutritive value could be obtained by in vivo digestibility trials and amino acid profile determination.

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