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Characterization of Seed Oil from Six In Situ Collected Wild *Amaranthus* Species

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Abstract: Six *Amaranthus* species (*A. cruentus*, *A. hybridus*, *A. hypochondriacus*, *A. muricatus*, *A. tuberculatus*, and *A. viridis*) were collected in Italy (wild habitats) from crops and roadsides. Amaranth seed oil was extracted to obtain fractions rich in squalene. Squalene, free fatty acid, tocopherol, and sterol composition and content were investigated in detail. An analysis of variance and principal components was performed. The oil content in the seed ranged from 5.17% (*A. muricatus*) to 12.20% (*A. tuberculatus*). The quantity of squalene in the oil varied from 3.43% (*A. muricatus*) to 6.09% (*A. hypochondriacus*). The primary sterols were beta-sitosterol, brassicasterol, campesterol, and stigmasterol. The main tocopherols in all the samples were alfa-tocopherol, beta-tocopherol, and delta-tocopherol. Our results exhibited that the smallest seeds (*A. tuberculatus*) have the highest percentages of oil and squalene, whereas the largest seeds size (*A. muricatus*) show the lowest percentages. There is also evidence that the samples growing at lower altitudes show the highest concentration of fatty acids. According to our results, the six wild *Amaranthus* species exhibited similar characteristics to commercial species. This study confirms that the site of the collection has an impact on the oil and squalene content of the *Amaranthus* species.

Keywords: *Amaranthus*; fatty acids; Italy; seeds; squalene; statistical analysis; tocopherols; sterols

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1. Introduction

The genus *Amaranthus* L. (Amaranthaceae Juss.) comprises 65–70 species, of which approximately half are native to the Americas [1]. Some taxa are used as ornamentals, food, and medicines, and they are able to spread due to cultivation, negatively impacting agricultural and natural ecosystems [1–3]. Taxonomically, this genus is complex due to its high phenotypic variability, which has resulted in the current nomenclatural disorders and misapplication of several names [4–9]. *Amaranthus* species have the C4 photosynthetic pathway, which allows them to proliferate at high temperatures and light levels, tolerate drought, and aggressively compete with warm-season plants for light, moisture, and nutritive substances. All these characteristics make Amaranth a valuable plant, particularly in parts of the world with scarce water resources [10]. The interest in Amaranth seeds, both for their use in the food field (human and animal nutrition) and their application in the industrial field, is increasing, mainly because its seeds are a source of minerals and vegetable oil rich in essential fatty acids, vitamins, and unsaponifiable substances, particularly squalene [11]. Squalene is a biochemical antecedent of sterols and occurs naturally as a triterpenoid. It has a significant role in medicine, cosmetics, and therapeutic applications. Recently, it has also had a significant role in drug design as an antioxidant and anticarcinogen [12]. Additionally, it is also used to reduce cholesterol levels among

human beings. It also shows promise as an excellent oxidation-resistant lubricant in the industrial sector [13].

In the past, the primary source of squalene was whale and shark liver, containing 40–80% squalene by weight. Due to marine animal protection concerns, researchers have turned their attention to exploring and identifying alternative affordable and sustainable sources of squalene, such as plants [14,15]. Among the different sources of squalene (yeast, olive oil, rice, corn, soy, peanuts, etc.) identified so far [13], Amaranth seeds are the most important and reliable source for their high squalene concentration (6–8%) compared to other sources [16,17]. The importance and value of squalene in cosmetics, pharmaceuticals, and the food industry allow advances in up-to-date technologies to purify squalene in higher concentrations from diverse resources [18]. Recently, new innovative and diverse eco-friendly technologies (thermal and non-thermal) have been developed and successfully applied in food technology. Next to conventional technologies for extracting essential and valuable bioactive compounds from plants, researchers have used high hydrostatic pressure (HHP), microwave (MW), ultrasound (US), infrared (IR), pulsed electric fields (PEF), and supercritical fluids [13].

Accelerated solvent extraction (ASE) is a helpful extraction technique that gives an excellent yield of extracted materials, involving the use of different organic solvents with varying polarity at elevated temperatures and pressure. This technique is time-saving and reduces solvent use [12,18,19]. Furthermore, it was discovered in a different study that ASE had the highest squalene yield, followed by Soxhlet and supercritical fluid extraction [18]. To extend the investigations of Amaranth seeds and broaden knowledge from a geographical point of view, it was decided to study *Amaranthus* species that grow spontaneously in Italy in different environments. Environmental factors such as climate, altitude, and soil pH should affect the chemical composition and squalene content [20]. In detail, the present paper aims to evaluate the chemical composition of seed oil extracted from six *Amaranthus* species collected in situ using the ASE apparatus. In particular, the squalene content, free fatty acid content, tocopherol, and sterol composition and content were investigated. This research aimed to understand the value of seed oils of wild species belonging to the genus *Amaranthus*, providing guidelines for future studies on food chemistry and industrial applications. Furthermore, the collected Amaranth seeds will be made accessible via GenBank to preserve the plant's genetic materials. Preserved seeds may be used in the future in experimental fields.

2. Materials and Methods

2.1. Plant Material

The research was based on our field investigations carried out in Italy in 2021. Six Amaranth species (*Amaranthus cruentus* L., *A. hybridus* L., *A. hypochondriacs* L., *A. muricatus* (Gillies ex Moq.) Hieron., *A. tuberculatus* (Moq.) J.D. Sauer., and *A. viridis* L.) were collected from wild fields in Italy (Table 1 and Figure S1). The species selected were determined primarily by the absence of data regarding seed oil and squalene in literature, and secondly by the rare or common status of the species in the Italian territory. For the common species *A. hybridus* and A. *hypochondriacus*, two and three collections, respectively, were made in NC, Italy, where these species are spread throughout the agricultural landscape.

The collected material was identified using the recent monograph on Italian *Amaranthus* by [1]. Around 40–50 individuals per site were collected in the fruiting stage. Synflorescences were later air-dried in the shade, and their fruits were subsequently separated and placed in closed containers. The voucher specimens were deposited at the Herbarium of the University of Molise IS (code according to [21] (Table 1). The seeds were deposited at the gene bank of the University of Molise. Three morphological characteristics were examined for each seed using a stereoscope (Leica MZ12): seed coat color, and the mean value of the length and width of the seeds.

Table 1. List of the collected specimens. Bioclimate is based on [22].

Amaranthus Species	Voucher Specimen	Italian Region (Code)	Coordinates	Altitude m (a.s.l.)	Date of Collection	Substrate	Bioclimate	Habitat
A. cruentus	12,002	Veneto (VEN)	45°17′49.2″ N 11°53′31.2″ E	5	21 October 2021	Clastic, soil with fertilizers	Temperate sub-continental	Cereals and vines crops
A. hybridus	12,005	Friuli Venezia Giulia (FVG)	46°03′14.4″ N 13°04′18.8″ E	144	8 August 2021	Clastic, soil with fertilizers	Temperate sub-continental	Crop in full sun
A. hybridus	12,006	Piedmont (PIE)	45°05′29.0″ N 7°22′53.3″ E	348	12 September 2021	Sandy, silty soil with coarse pebble component deriving from river flooding	Temperate semi-continental	Abandoned garden in the alluvial plain
A. hybridus	12,007	Veneto (VEN)	46°06′39.2″ N 12°08′20.4″ E	380	17 September 2021	Calcareous matrix	Temperate semi-continental	Corn crop
A. hypochondriacus	12,008	Veneto (VEN)	46°07′60.0″ N 12°15′32.5″ E	440	22 September 2021	Calcareous matrix	Temperate semi-continental	Corn crop
A. hypochondriacus	12,009	Lazio (LAZ)	41°49′44.4″ N 13°08′24.0″ E	625	15 October 2021	Soil rich in nitrogen	Temperate oceanic	Roadside
A. muricatus	12,004	Molise (MOL)	42°00′14.4″ N 14°59′45.6″ E	14	17 July 2021	Calcareous matrix	Mediterranean oceanic	Stony wall of the Svevo castle
A. tuberculatus	12,001	Marche (MAR)	43°48′39.0″ N 13°02′21.0″ E	5	23 September 2021	Terrigenous matrix	Transitional semi-continental	Gravelly riverbed, in full sun
A. viridis	12,003	Campania (CAM)	40°51′07.2″ N 14°16′22.8″ E	12	21 July 2021	Soil rich in nitrogen	Mediterranean oceanic	Roadside

2.2. Oil Extraction and Oil Content Determination

The *Amaranthus* seeds were cleaned and oven-dried (2 h in a vacuum oven at 45 °C). Afterward, seeds were packed in airtight containers with cotton and silica gel and stored in a cold room until use. The cleaned and oven-dried seeds (50 g) were then powdered using a Fritsch Pulverisette 6 planetary mono mill (400 rpm for 4–8 min, depending on the species), excepting *Amaranthus viridis* L., which took 15 min to grind. The process of extraction was performed using an ASE350 apparatus (Dionex). A 22 mL extraction cell was filled with a mixture of 8–13 g milled seeds and 1.1 g of celite powder. The extraction solvent being used was n-hexane (100%), and three extraction cycles were performed during the process. The optimized conditions used for extraction included a temperature of 70 °C and static time of 10 min. As the extraction was completed, the excess solvent was evaporated using a rotary evaporator (Büchi). The pre-set temperature for the water bath was 60 °C, and the vacuum was set at 370 mbar. After evaporation, the mixture was left overnight in the refrigerator in a fume hood to allow any residual solvent (n-hexane) to be removed. Finally, the mass of oil was recorded.

2.3. Determination of Squalene, Free Fatty Acid, Tocopherol, and Sterol Content

The squalene, free fatty acids, tocopherol, and sterol content in the oil was determined by gas chromatography (Trace 1300 Thermo Scientific Interscience, Louvain-la-Neuve, Belgium) using a capillary MXT-5 column (30 m × 0.53 mm, film thickness 0.25 µm, Restek, Interscience, Louvain-la-Neuve, Belgium) equipped with an SSL injector (inlet temperature 350 °C) and with a flame ionization detector (GC-FID) (380 °C, constant flow, hydrogen 35 mL/min, air 350 mL/min, nitrogen 40 mL/min) under a temperature gradient (50 °C for 1 min, ramp to 180 °C at 10 °C/min; ramp to 230 °C at 3 °C/min; ramp to 380 °C at 15 °C/min; 380 °C for 10 min). A constant helium flow of 1.5 mL/min was applied as a carrier gas. Samples for GC analysis were prepared by mixing 0.1 g of a 10 m% oil solution in n-heptane with 0.1 g of 0.1 m% tetradecane solution in n-heptane (internal standard solution) and diluting up to 1 g with n-heptane. A calibration was performed using squalene, fatty acid, tocopherol, and sterol standards (Acros Organics) to perform a quantitative analysis. Analysis results were evaluated using Chromeleon 6 software [23].

2.4. Statistical Analysis

All the experiments in this study were conducted in triplicate, and the data were reported as the mean ± standard deviation (SD). Principal component analysis (PCA) was performed using PAST 4.11 [24]. The data were subjected to statistical analysis using XLSTAT software version 2021.5.1 [25]. Analysis of variance (ANOVA) and Tukey's HSD

multiple comparison tests were used to identify significant differences among the different samples at $p \leq 0.05$. Data are available within the article and in Supplementary Materials.

3. Results and Discussion

3.1. Seed Features

The sizes of the nine *Amaranthus* samples investigated exhibited mean values ranging from the minimum values in the length of 0.399 mm and width of 0.385 mm (*A. tuberculatus* MAR) to the maximum values in the length of 0.642 mm and width of 0.589 mm (*A. muricatus* MOL) (Table 2). In detail, *A. cruentus* VEN and *A. tuberculatus* MAR were statistically different ($p < 0.001$) from each other and from all the other samples. It should be noted that *A. hypocondriacus* and *A. hybridus*, collected in different stands, showed similar values in order of magnitude. The seed color was quite consistent for the Amaranth samples collected in different regions: *A. hybridus* seeds were black while *A. hypocondriacus* seeds were dark brown (Table 2). The seed color was also black for *A. cruentus*, *A. muricatus*, and *A. virdis*, and dark brown for *A. tuberculatus* (Table 2).

Table 2. Compositional characteristics of seeds of the six *Amaranthus* species collected from Italian wild habitats: SDO, seed color; SL, average seed length (mm); SW, average seed width (mm); OYS, average oil yield in seeds (%); CSO, average concentration squalene in oil (%); CSS, average concentration squalene in seeds (%); FAS, average free fatty acids in seed oil (%); SSO, average sterols in seed oil (ppm); TSO, average tocopherols in seed oil (ppm). Different letters (a–e) indicate significant differences according to Tukey's HSD test ($p < 0.05$). **, *** represent significant at $p < 0.01$, 0.001, respectively. n.s.: not significant.

***Amaranthus* Species**	**SDO**	**SL**	**SW**	**OYS**	**CSO**	**CSS**	**FAS**	**SSO**	**TSO**
A. cruentus (VEN)	Black	0.476 ^{b} ± 0.030	0.436 ^{b} ± 0.018	6.29 ^{ab} ± 1.08	5.94 ^{b} ± 0.87	0.37 ^{abc} ± 0.03	1.73 ^{a} ± 0.28	208 ^{ab} ± 135	152 ± 71
A. hybridus (FVG)	Black	0.585 ^{cde} ± 0.014	0.494 ^{cd} ± 0.018	7.47 ^{ab} ± 1.25	4.94 ^{ab} ± 0.72	0.36 ^{abc} ± 0.02	0.39 ^{a} ± 0.30	450 ^{ab} ± 309	327 ± 238
A. hybridus (PIE)	Black	0.561 ^{cd} ± 0.008	0.483 ^{c} ± 0.016	9.53 ^{ab} ± 1.59	4.75 ^{ab} ± 0.40	0.45 ^{bc} ± 0.03	0.66 ^{a} ± 0.48	147 ^{a} ± 14	70 ± 27
A. hybridus (VEN)	Black	0.548 ^{c} ± 0.004	0.480 ^{c} ± 0.005	6.05 ^{ab} ± 0.57	5.78 ^{ab} ± 1.50	0.35 ^{ab} ± 0.09	0.65 ^{a} ± 0.31	361 ^{ab} ± 299	214 ± 133
A. hypochondriacus (VEN)	Dark brown	0.576 ^{cd} ± 0.015	0.534 ^{d} ± 0.022	9.77 ^{ab} ± 0.89	6.09 ^{b} ± 0.75	0.59 ^{c} ± 0.02	0.84 ^{a} ± 0.67	219 ^{ab} ± 72	140 ± 54
A. hypochondriacus (LAZ)	Dark brown	0.621 ^{de} ± 0.040	0.579 ^{e} ± 0.014	6.64 ^{ab} ± 3.12	4.01 ^{ab} ± 0.80	0.25 ^{ab} ± 0.06	0.65 ^{a} ± 0.05	857 ^{b} ± 492	149 ± 8
A. muricatus (MOL)	Black	0.642 ^{e} ± 0.006	0.589 ^{e} ± 0.021	5.17 ^{a} ± 1.45	3.43 ^{a} ± 0.30	0.17 ^{a} ± 0.03	1.17 ^{a} ± 1.04	268 ^{ab} ± 44	536 ± 212
A. tuberculatus (MAR)	Dark brown	0.399 ^{a} ± 0.016	0.385 ^{a} ± 0.012	12.20 ^{b} ± 4.74	4.94 ^{ab} ± 0.573	0.59 ^{c} ± 0.18	2.42 ^{a} ± 2.02	83 ^{a} ± 94	223 ± 153
A. viridis (CAM)	Black	0.550 ^{c} ± 0.035	0.517 ^{cd} ± 0.024	6.24 ^{ab} ± 1.62	3.51 ^{a} ± 0.03	0.22 ^{ab} ± 0.05	8.93 ^{b} ± 2.17	136 ^{a} ± 137	333 ± 231
p-Value		***	***			**	.***		n.s.

3.2. Oil Content

In accordance with our findings, the oil content in the seeds (OYS) of the six *Amaranthus* species ranges from 5.17% for *A. muricatus* to 12.20% for *A. tuberculatus* (Table 2 and Figure 1), and these two species are significantly different from each other. In detail, *A. hybridus* gathered from three different regions in northern Italy (PIE, FVG, VEN; see Table 1 for regional codes) shows overall high values of oil content, with no statistically significant differences in the three stands: 9.53%, 7.47%, and 6.05% values, respectively. *A. hypochondriacus* collected from two different regions reveals divergent values of 9.77% in the Veneto region (north Italy) and 6.64% in the Lazio region (central Italy), but these values are not statistically significant. Lastly, the oil content of *A. cruentus* is 6.29%, whereas it is 6.24% for *A. viridis* (Table 2 and Figure 1). According to our research, smaller-sized seeds (*A. tuberculatus* MAR) contain the highest percentage of oil, whereas larger seeds

(*A. muricatus* MOL) contain the lowest percentage of oil OYS and squalene CSO. The OYS percentages obtained in this work support those previously reported: they show that the OYS in *A. cruentus* ranges from 1.09% to 8.20%, and from 3.03% to 8.70% in *A. hypochondriacus* ranges [26–28].

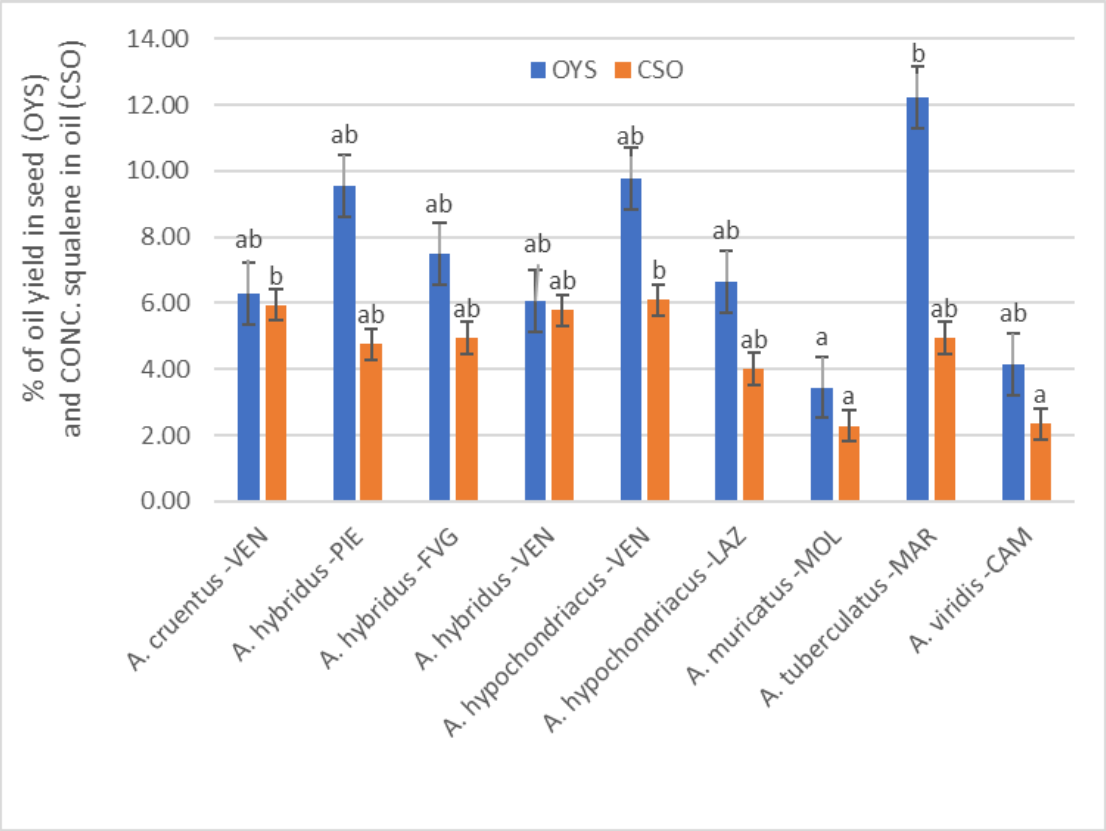

Figure 1. Percentage of oil yield in seed (OYS) and concentration of squalene in oil (CSO) for the six species of *Amaranthus* collected. Different letters (a–d) indicate significant differences according to Tukey's HSD test ($p < 0.05$).

3.3. Squalene Content

The investigation of the six *Amaranthus* species reveals considerable variations in the squalene content (CSO) depending on the samples (Table 2 and Figure 1). As a whole, the range of CSO varies from 3.43% for *A. muricatus* to 6.09% for *A. hypochondriacus* VEN (Tables 1 and 2, and Figure 1). In detail, the species that grow at low altitudes and in a Mediterranean bioclimate (*A. muricatus*, *A. viridis*) show the significantly lowest CSO values (3.43%, 3.51%). On the other hand, *A. hypochondriacus* VEN and *A. cruentus* VEN, which grow in a temperate bioclimate, show the significantly highest CSO values (6.09% and 5.94%) (Tables 1 and 2, and Figure 1). The CSO values for *A. hybridus* are very similar (PIE: 4.75%, FVG: 4.94%, VEN: 5.78%).

The results obtained for wild species are consistent with previously published results on squalene content in cultivated Amaranth species (Table 3). Regarding *A. cruentus*, *A. hypocondriacus*, and *A. hybridus*, our research results confirm the range of the values and align with the highest values found in the literature [14,26–33]. For *A. muricatus*, *A. tuberculatus*, and *A. viridis*, our research found values comparable to those reported in [14], and also contribute to the limited knowledge of CSO for these three species.

3.4. Free Fatty Acid, Sterol, and Tocopherol Content

Our results show a strongly varying amount of free fatty acids (FAS) found in *Amaranthus* oil for all the samples. The free fatty acid range is from 0.39% for *A. hybridus* FVG

to 8.93% for *A. viridis* CAM (Table 2). *A. cruentus*, *A. muricatus*, *A. tuberculatus*, and *A. viridis*, collected at low altitudes (5 m, 14 m, 5 m, 12 m a.s.l.), show high levels of free fatty acids (1.73%, 1.17%, 2.42%, 8.93%), and only the value of A. *viridis* is significantly different from all the others (Table 2). Instead, the species growing at medium and high altitudes (144 m, 348 m, 380 m, 440 m, and 625 m a.s.l.), namely *A. hybridus* FVG, *A. hybridus* PIE, *A. hybridus* VEN, *A. hypochondriacus* VEN, and *A. hypochondriacus* LAZ, show free fatty acids levels lower than 1 (0.39%, 0.66%, 0.65%, 0.84%, and 0.65% respectively). The free fatty acid composition shows the nutritional value and stability of fats and oils. Our findings support the earlier studies that suggested Amaranth oil is a reliable source of free fatty acids [14,27,28,31,34–37].

Table 3. Comparison between squalene content in oil CSO from this study and from the major references data.

***Amaranthus* Species**	**CSO**	**Reference**
A. cruentus	5.94	Current study
	6.96	Lyon et al., 1987 [33]
	6.56	León-Camacho et al., 2001 [32]
	4.2–5.44	He et al., 2002 [28]
	2.26–5.94	Bergenza et al., 2003 [29]
	3.32–4.93	He et al., 2003 [14]
	4.9	Gamel et al., 2007 [30]
	5.29–6.25	Bozorov et al., 2018 [26]
	5.74–6.95	El Gendy et al., 2018 [31]
A. hybridus	4.75–5.78	Current study
	5.23	He et al., 2002 [28]
	2.26–7.3	He et al., 2003 [14]
A. hypochondriacus	4.01–6.09	Current study
	3.62–5.01	He et al., 2002 [28]
	4.74–6.98	He et al., 2003 [14]
	6.05–7.12	Bozorov et al., 2018 [26]
A. muricatus	3.43	Current study
	3.20	He et al., 2003 [14]
A. tuberculatus	4.94	Current study
	4.75	He et al., 2003 [14]
A. viridis	3.51	Current study
	3.28–5.74	He et al., 2003 [14]

Our research indicates that beta-sitosterol, brassicasterol, campesterol, and stigmasterol are the major sterols (SSO) present in *Amaranthus* oil (Table S1). Additionally, beta-sitosterol is the principal sterol present in all the Amaranth samples. The total sterols (SSO) range is between 83 ppm for *A. tuberculatus* and 857 ppm for *A. hypochondriacus* LAZ (Table 2). According to our study, the ranges of major sterols are 47–738 ppm for beta-sitosterol, 12–43 ppm for brassicasterol, 2–9 ppm for campesterol, and 8–130 ppm for stigmasterol (Table S1). Our results are similar to those of prior publications [38,39]. It is interesting to note that, among the Amaranth samples, the total sterol content (SSO) of *A. hypochondriacus* (LAZ) and A. hybridus (FVG and VEN) (Table 2) is higher than that of other plants studied, such as olive, peanut, palm, coconut, walnut, cashew, and almond [39,40].

Based on our studies, the range for total tocopherols (TSO) is from 70 ppm for the *A. hybridus* PIE region to 536 ppm for *A. muricatus* (Table 2). The ranges for the three main tocopherols are 6–14 ppm for alfa-tocopherol, 11–158 ppm for beta-tocopherol, and 39–289 ppm for delta-tocopherol (Table S2). These ranges are comparable to other results stated in the literature [38,41,42].

3.5. Multivariate Analysis

In the principal component analysis (Figure 2), the cumulative percentage of the first two axes (Components 1 and 2) is 75.464%, with the first one (PC1) contributing 52.039%

to the total variance and the second (PC2) contributing 23.425%. A positive correlation between seed length (SL), seed width (SW), sterols in seed oil (SSO), and tocopherols in seed oil (TSO) was found along PC1. Conversely, the correlation is negative between the oil yield in seed (OYS), the concentration of squalene in seed oil (CSO), and the concentration of squalene in seed (CSS). PC2 shows a positive correlation with altitude (ALT) and a negative correlation with fatty acids (FAS).

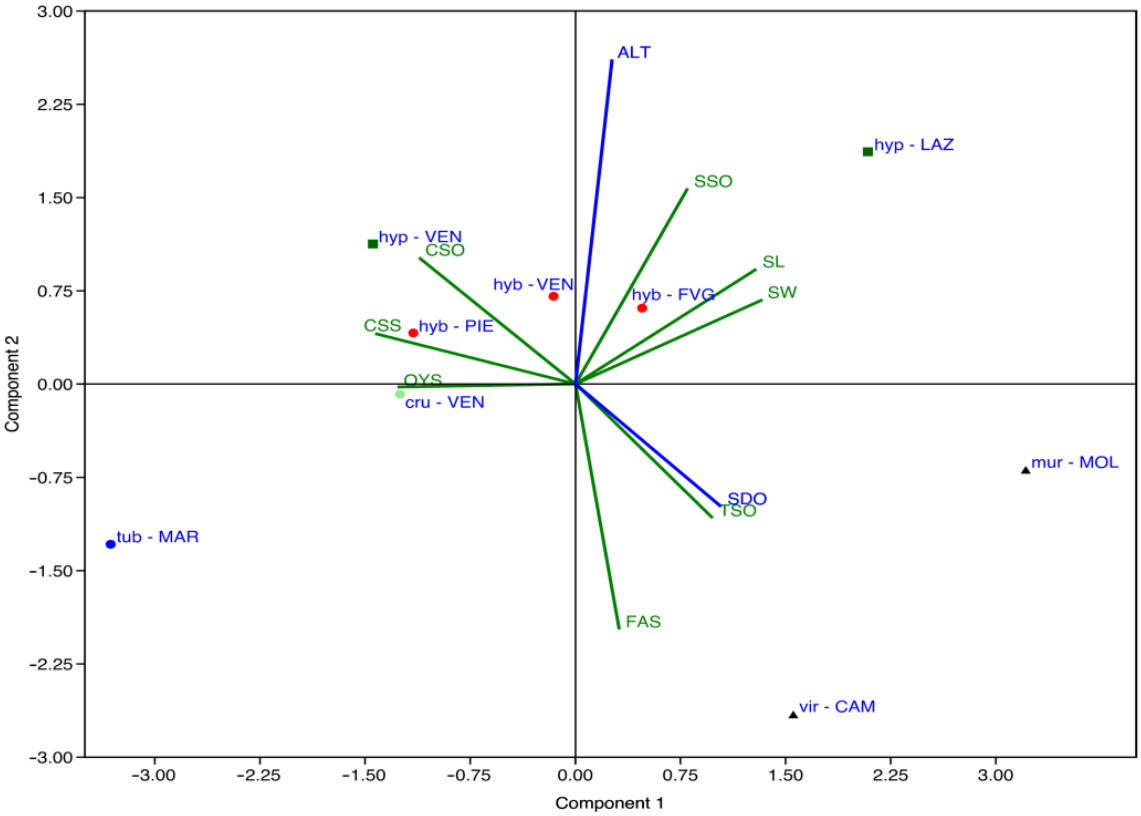

Figure 2. Scatterplot of the first two principal components from the PCA was carried out with 10 variables and 9 samples. Blue line: ALT (altitude); SDO (seed color); green line: OYS (oil yield in seed); CSO (conc. squalene in oil); CSS (conc. squalene in seed); TSO (tocopherols in seed oil); SSO (sterols in seed oil); FAS (free fatty acids in seed oil); SL (seed length); SW (seed width).

PCA confirms the results obtained from the previous analysis: large seeds exhibit a higher tocopherol (TSO) and sterol (SSO) content, whereas small seeds exhibit a higher amount of oil yield in seed (OYS), concentration of squalene in seed oil (CSO), and concentration of squalene in seed (CSS). *A. hybridus* and *A. hypocondriacus* collected from altitudes from 144 to 625 m have low values of fatty acids (FAS); conversely, the species *A. muricatus*, *A. tuberculatus*, and *A. viridis* collected at low altitudes (5–14 m) exhibit the opposite behavior.

4. Conclusions

This study provides, for the first time, data on the squalene, free fatty acid, tocopherol, and sterol content and composition of six *Amaranthus* species that grow in wild habitats. These species exhibited similar characteristics to the commercial species, with both showing medium–high values for oil and squalene content. This study confirms that the collection site influences the oil and squalene content of the *Amaranthus* species. The growing interest in the seed of the genus *Amaranthus* all over the world, such as in nutraceutical, industrial, and medical fields, makes it necessary to expand the spectrum of the Amaranth species to be cultivated as a good source of squalene and other components.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15020237/s1, Figure S1: regions where the *Amaranthus* species were collected in Italy: CAM Campania, FVG Friuli-Venezia Giulia, LAZ Lazio, MAR Marche, MOL Molise, PIE Piedmont, VEN Veneto. Other regions: AOS Val d'Aosta, LOM Lombardia, TRA Trentino-Alto Adige, LIG Liguria, EMR Emilia-Romagna, SMR Republic of San Marino, TOS Toscana, UMB Umbria, SCV Vatican City, State ABR Abruzzo, PUG Puglia, BAS Basilicata, CAL Calabria, SAR Sardegna, SIC Sicilia; Table S1: Sterol (beta-sitosterol, brassicasterol, campesterol, stigmasterol) composition of Amaranth seed oil collected from Italian wild habitats; AVG: average value, SD: standard deviation; Table S2: Tocopherol (alfa-tocopherol, beta-tocopherol, delta-tocopherol) composition of Amaranth seed oil collected from Italian wild habitats; AVG: average value, SD: standard deviation.

Author Contributions: Conceptualization, A.N.H. and P.F.; methodology, A.N.H., J.G., M.M. and A.V; software, A.N.H. and P.D.M.; validation, P.F. and A.N.H.; formal analysis, A.N.H. and J.G.; investigation, A.N.H. and P.F.; resources, A.N.H., J.G., M.M. and A.V.; data curation, P.F. and J.G.; writing—original draft preparation, A.N.H.; writing, A.N.H., J.G., M.M., A.V., D.I. and P.D.M.; review and editing, P.F., D.I. and J.G.; supervision, P.F. All authors have read and agreed to the published version of the manuscript.

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References

1. Iamonico, D. Taxonomic revision of the genus *Amaranthus* (Amaranthaceae) in Italy. *Phytotaxa* **2015**, *199*, 1–84. [CrossRef]
2. Das, S. *Amaranthus: A Promising Crop of the Future*; Springer Nature: Singapore, 2016; pp. 13–48. [CrossRef]
3. Iamonico, D. II Genere *Amaranthus* L. in Italia: Stato attuale delle conoscenze. *Ann. Missouri Bot. Gard* **2010**, *273*, 149–154. [CrossRef]
4. Bayón, N.D. Revisión Taxonómica de las Especies Monoicas de *Amaranthus* (Amaranthaceae): *Amaranthus* subg. *Amaranthus Amaranthus subg. Albersia1. Ann. Mo. Bot. Gard.* **2015**, *101*, 261–383. [CrossRef]
5. Iamonico, D. Nomenclatural survey of the genus *Amaranthus* (Amaranthaceae). 4. Detailed questions arising around the name *Amaranthus gracilis*. *Bot Serb* **2016**, *40*, 61–68. [CrossRef]
6. Iamonico, D. Nomenclature survey of the genus *Amaranthus* (Amaranthaceae). 5. Moquin-Tandon's names. *Phytotaxa* **2016**, *273*, 81–114. [CrossRef]
7. Iamonico, D. A nomenclatural survey of the genus *Amaranthus* (Amaranthaceae) 7: Names published by Willdenow. *Willdenowia* **2020**, *50*, 147–155. [CrossRef]
8. Iamonico, D.; El Mokni, R. *Amaranthus palmeri* (Amaranthaceae) in Tunisia, a second record for the continental African flora and nomenclatural notes on *A. sonoriensis* nom. nov. pro *A. palmeri* var. *glomeratus*. *Bothalia* **2017**, *47*, 4. [CrossRef]
9. Iamonico, D.; Palmer, J. Nomenclature survey of the genus *Amaranthus* (Amaranthaceae). 6. Names linked to the Australian flora. *Aust. Syst. Bot.* **2020**, *33*, 169–173. [CrossRef]
10. Nogoy, K.M.; Yu, J.; Song, Y.G.; LI, S.; Chung, J.W.; Choi, S.H. Evaluation of the nutrient composition, in vitro fermentation characteristics, and in situ degradability of *Amaranthus caudatus*, *Amaranthus cruentus*, and *Amaranthus hypochondriacus* in Cattle Animals. *Basel* **2021**, *11*, 18. [CrossRef]
11. Sayed-Ahmad, B.; Urrutigoïty, M.; Hijazi, A.; Saad, Z.; Cerny, M.; Evon, P.; Talou, T.; Merah, O. Amaranth oilseed composition and cosmetic applications. *Separations* **2022**, *9*, 181. [CrossRef]
12. Sheng, Y.Y.; Xiang, J.; Wang, K.R.; Li, Z.Y.; Li, K.; Lu, J.L.; Ye, J.H.; Liang, Y.R.; Zheng, X.Q. Extraction of squalene from tea leaves (*Camellia sinensis*) and its variations with leaf maturity and tea cultivar. *Front. Nutr.* **2022**, *9*, 37. [CrossRef]
13. Wejnerowska, G.; Heinrich, P.; Gaca, J. Separation of squalene and oil from *Amaranthus* seeds by supercritical carbon dioxide. *Sep. Purif. Technol.* **2013**, *110*, 39–43. [CrossRef]
14. He, H.-P.; Corke, H. Oil and squalene in *Amaranthus* grain and leaf. *Agric. Food Chem.* **2003**, *51*, 7913–7920. [CrossRef]
15. Sun, H.; Wiesenborn, D.; Tostenson, K.; Gillespie, J.; Rayas-Duarte, P. Fractionation of squalene from amaranth seed oil. *JAOCS* **1997**, *74*, 413–418. [CrossRef]
16. Aderibigbe, O.R.; Ezekiel, O.O.; Owolade, S.O.; Korese, J.K.; Sturm, B.; Hensel, O. Exploring the potentials of underutilized grain amaranth (*Amaranthus* spp.) along the value chain for food and nutrition security: A review. *Crit. Rev. Food Sci. Nutr.* **2022**, *62*, 656–669. [CrossRef]

17. Taia, W.K.; Shehata, A.A.; Elshamy, E.M.; Ibrahim, M.M. Biosystematic studies for some Egyptian *Amaranthus* L. taxa and their significance in their identification. *Taeckholmia* **2020**, *40*, 85–99. [CrossRef]
18. Krulj, J.; Brlek, T.; Pezo, L.; Brkljača, J.; Popović, S.; Zeković, Z.; Bodroža Solarov, M. Extraction methods of *Amaranthus* sp. grain oil isolation. *J. Sci. Food Agric.* **2016**, *96*, 3552–3558. [CrossRef]
19. Sun, H.; Ge, X.; Lv, Y.; Wang, A. Application of accelerated solvent extraction in the analysis of organic contaminants, bioactive and nutritional compounds in food and feed. *J. Chromatogr.* **2012**, *1237*, 1–23. [CrossRef]
20. Thomas, W.E.; Burke, I.C.; Spears, J.F.; Wilcut, J.W. Influence of environmental factors on slender amaranth (*Amaranthus viridis*) germination. *Weed Sci.* **2006**, *54*, 316–320. [CrossRef]
21. Di Marzio, P.; Fortini, P. (Eds.) *Il Museo Erbario dell'Università del Molise*; Università degli Studi del Molise: Campobasso, Italy, 2015; p. 38. ISBN 9788896394151. Available online: https://hdl.handle.net/11695/47228 (accessed on 22 November 2022).
22. Blasi, C.; Capotorti, G.; Copiz, R.; Guida, D.; Mollo, B.; Smiraglia, D.; Zavattero, L. *Terrestrial Ecoregions of Italy*; Map and Explanatory Notes; Global Map s.r.l., S.EL.C.A.: Firenze, Italy, 2018; p. 36. Available online: https://hdl.handle.net/11386/4746968 (accessed on 22 November 2022).
23. Szabóová, M.; Záhorský, M.; Gažo, J.; Geuens, J.; Vermoesen, A.; D'Hondt, E.; Hricová, A. Differences in seed weight, amino acid, fatty acid, oil, and squalene content in γ-irradiation-developed and commercial amaranth varieties (*Amaranthus* spp.). *Plants* **2020**, *9*, 1412. [CrossRef]
24. Addinsoft, A. *Addinsoft XLSTAT Statistical and Data Analysis Solution*; Long Island: New York, NY, USA, 2023.
25. Hammer, Ø.; Harper, D.A.T.; Ryan, P.D. PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontol. Electron.* **2001**, *4*, 9.
26. Bozorov, S.S.; Berdiev, N.S.; Ishimov, U.J.; Olimjonov, S.S.; Ziyavitdinov, J.F.; Asrorov, A.M.; Salikhov, S.I. Chemical composition and biological activity of seed oil of amaranth varieties. *Nova Biotechnol. Chim.* **2018**, *12*, 66–73. [CrossRef]
27. Hlinková, A.; Bednarova, A.; Havrlentová, M.; Šupová, J.; Čičová, I. Evaluation of fatty acid composition among selected amaranth grains grown in two consecutive years. *Biologia* **2013**, *68*, 641–650. [CrossRef]
28. He, H.P.; Cai, Y.; Sun, M.; Corke, H. Extraction and purification of squalene from *Amaranthus* grain. *J. Agric. Food Chem.* **2002**, *50*, 368–372. [CrossRef]
29. Berganza, B.E.; Moran, A.W.; Rodríguez, G.M.; Coto, N.M.; Santamaría, M.; Bressani, R. Effect of variety and location on the total fat, fatty acids and squalene content of amaranth. *Plant Foods Hum. Nutr.* **2003**, *58*, 1–6. [CrossRef]
30. Gamel, T.H.; Mesallam, A.S.; Damir, A.A.; Shekib, L.A.; Linssen, J.P. Characterization of amaranth seed oils. *J. Food Lipids* **2007**, *14*, 323–334. [CrossRef]
31. El Gendy, A.N.G.; Tavarini, S.; Conte, G.; Pistelli, L.; Hendawy, S.F.; Omer, E.A.; Angelini, L.G. Yield and qualitative characterisation of seeds of *Amaranthus hypochondriacus* L. and *Amaranthus cruentus* L. grown in central Italy. *IJA* **2018**, *13*, 63–73. [CrossRef]
32. León-Camacho, M.; García-González, D.L.; Aparicio, R. A detailed and comprehensive study of amaranth (*Amaranthus cruentus* L.) oil fatty profile. *Eur. Food Res. Technol.* **2001**, *213*, 349–355. [CrossRef]
33. Lyon, C.K.; Becker, R. Extraction and refining of oil from amaranth seed. *JAOCS* **1987**, *64*, 233–236. [CrossRef]
34. Martirosyan, D.M.; Miroshnichenko, L.A.; Kulakova, S.N.; Pogojeva, A.V.; Zoloedov, V.I. Amaranth oil application for coronary heart disease and hypertension. *Lipids Health Dis.* **2007**, *6*, 44–45. [CrossRef]
35. Ayorinde, F.O.; Ologunde, M.O.; Nana, E.Y.; Bernard, B.N.; Afolabi, O.A.; Oke, O.L.; Shepard, R.L. Determination of fatty acid composition of *Amaranthus* species. *JAOCS* **1989**, *66*, 1812–1814. [CrossRef]
36. Prakash, D.; Pal, M. Seed protein, fat and fatty acid profile of *Amaranthus* species. *J. Sci. Food Agric.* **1992**, *58*, 145–147. [CrossRef]
37. Srivastava, S.; Sreerama, Y.N.; Dharmaraj, U. Effect of processing on squalene content of grain amaranth fractions. *J. Cereal Sci.* **2021**, *100*, 103218. [CrossRef]
38. Nasirpour-Tabrizi, P.; Azadmard-Damirchi, S.; Hesari, J.; Piravi-Vanak, Z. Amaranth seed oil composition. In *Nutritional Value of Amaranth*; Intech Open: London, UK, 2020. [CrossRef]
39. Marcone, M.F.; Kakuda, Y.; Yada, R.Y. Amaranth as a rich dietary source of β-sitosterol and other phytosterols. *Plant Foods Hum. Nutr.* **2003**, *58*, 207–211. [CrossRef]
40. Phillips, K.M.; Ruggio, D.M.; Toivo, J.I.; Swank, M.A.; Simpkins, A.H. Free and esterified sterol composition of edible oils and fats. *J. Food Compos. Anal.* **2002**, *15*, 123–142. [CrossRef]
41. Kraujalis, P.; Venskutonis, P.R. Supercritical carbon dioxide extraction of squalene and tocopherols from amaranth and assessment of extracts antioxidant activity. *J. Supercrit. Fluids* **2013**, *80*, 78–85. [CrossRef]
42. Santiago, P.; Tenbergen, K.; Vélez-Jiménez, E.; Cardador-Martínez, M.A. Functional attributes of amaranth. *Int. J. Food Sci. Nutr.* **2014**, *2*, 1010.