Some Compositional Properties of Seeds and Oils of Eight *Amaranthus* Species

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ABSTRACT: Grain of 21 *Amaranthus* accessions (eight species) was analyzed for crude fat, fatty acid profiles (FAP), and vitamin E (tocopherols and tocotrienols). Contents of (1 → 3), (1 → 4) β-glucan were determined in 12 accessions (four species), and trypsin inhibitor activity (TIA) in 20 accessions (six species). FAP and vitamin E profiles were compared to those of barley, buckwheat, corn, lupin, oat, and wheat oils. Crude fat content ranged from 5.2 to 7.7%, and of the oils examined, amaranth oil was most similar in FAP to corn and buckwheat oils. Amaranth was higher than all but wheat and lupin in tocopherol content but was virtually devoid of tocotrienols, which have been shown to have hypocholesterolemic activity. Amaranth grain did not contain (1 → 3), (1 → 4) β-glucans and was low in trypsin inhibitor activity (≤4.3 trypsin units inhibited/mg). Any hypocholesterolemic effects of dietary amaranth are apparently due to substances other than (1 → 3), (1 → 4) β-glucans or tocotrienols.

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The genus *Amaranthus* includes more than 60 species that grow in many areas of the world, including Central and South America, Africa, India, and China (1). Amaranth is a dicotyledonous plant and is considered a pseudocereal, as are other edible seeds of dicots, such as buckwheat (*Fagopyrum esculentum*) and quinoa (*Chenopodium quinoa*) (2). This ancient crop originated near Tehaucan Puebla, Mexico, possibly around 4000 BC (3,4). Amaranth has been consumed for centuries as both a green leafy vegetable and as a grain, although there currently appears to be little commercialization of vegetable amaranth products (5).

The lipid content of amaranth seeds is typically between 4.8 and 8.1% (1), although *A. spinosus* and *A. tenuifolius* are reported to contain as much as 17.0 and 19.3%, respectively (6,7). The fatty acid profile (FAP) of amaranth oil is similar to that of corn oil and is not considered to be unique (8). Amaranth seed oil contains 2.4 to 8.0% squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene), a relatively high concentration (7–9). Squalene is an expensive terpenoid compound, derived primarily from shark and whale liver oils, and is used as an ingredient in cosmetics, skin penetrants, and lubricants for computer disks (10).

Amaranth oil is reported to contain relatively high concentrations of tocotrienols (11). These rare forms of vitamin E have been shown to inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase, the key regulatory enzyme in cholesterol biosynthesis (12). Pettersson and Aman (13) concluded that, although tocotrienols may influence cholesterogenesis, this effect is relatively small in chickens in comparison to the cholesterol-lowering properties of soluble dietary fibers, e.g., mixed linkage β-glucans or other unidentified factors. Diets containing amaranth grain were found to lower blood serum cholesterol levels in rats (14,15) and chicks (16). In one rat-feeding study (14), the authors noted that amaranth grain induced a hypocholesterolemic response similar to that of the soluble fiber in oat bran or pectin, but that the grain was low in soluble fiber. In the other rat-feeding study (15), the authors postulated that the hypocholesterolemic effect could be attributed to the preponderance of unsaturated fatty acids in amaranth seeds. In the chick-feeding study (16), low-density lipoprotein levels varied inversely with the tocotrienol content of the amaranth component of the diets, while high-density lipoprotein levels were largely unaffected. Laovoravit *et al.* (17) reported no significant difference in cholesterol contents of the livers of chicks that were fed diets containing either 30% raw amaranth or corn. Cholesterol biosynthesis is not controlled by a single factor and may also be influenced by the saturation/unsaturation (S/U) ratio of the dietary fatty acids (18), by dietary fiber, e.g., β-glucans (19), and possibly by squalene (12). The active hypocholesterolemic component(s) of amaranth grain and the mechanism(s) involved have not been clearly identified; the subject merits further research.

There have been conflicting reports about the presence and nature of antinutritional substances in amaranth grain. Although the nutritional scores of amaranth protein are routinely higher than those for cereal and legume proteins, this is not reflected in protein efficiency ratios (20). Moist heating of raw amaranth grain improved its protein quality in all species tested (21,22). When processing is controlled so as to mini-

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mally alter the availability of essential amino acids, amaranth protein quality approaches that of casein (23). The trypsin inhibitor in *A. hypochondriacus* has been purified and characterized, and the complete amino acid sequence has been determined (24). Levels of trypsin inhibitors and lectins in amaranth grain have been reported to be low (25,26) and at levels unlikely to present a nutritional hazard (7). On the other hand, antiphysiological factors can vary greatly among species, origin, and variety (27). Antinutrients in one amaranth cultivar apparently impeded the growth of rats (28). Much of the published research on trypsin inhibitor activity (TIA) in amaranth grain has focused on a single species, cultivar, or accession (25,26).

The objectives of this study were to analyze a fairly broad range of Minnesota-grown amaranth grain, including eight different species and several accessions within the more popular species, for crude fat content, fatty acid, and vitamin E (tocols) profiles, (1 → 3), (1 → 4) β-glucan content, and TIA. The fatty acid and vitamin E profiles of barley, buckwheat, corn, lupin, oat, and wheat oils were also determined for comparison and to validate the methodology. The results should be useful to nutritionists concerned with cholesterol metabolism and to plant breeders interested in genetically modifying the composition of the grain.

MATERIALS AND METHODS

Amaranth and other grain seeds. All accessions of amaranth, except AAI 1492, which was obtained from Dr. James Lehmann, were grown in 1992 on field plots at Rosemount, MN, which is at about 45° north latitude. The soil is characterized as Waukegan silt loam (fine-silty over sandy or sandy-skeletal, mixed, mesic Typic Hapludoll). The growing season was considered normal in temperature and higher than normal in precipitation for this region.

Over 100 accessions of amaranth, representing 23 cultivars from around the world, were densely planted into 76-cm rows in late May. The plants were hand-thinned to approximately 6 cm between plants at the four-leaf stage. Weed control consisted of hand cultivation, and no herbicides or insecticides were used. Phosphorus and potassium were added according to soil tests. Nitrogen as ammonium nitrate (NH_4NO_3) was added at the rate of 85 kg N/ha. The crops were harvested in mid-October, approximately eight days after the first killing frost. A total of 26 accessions were selected for analytical testing based on the criteria of seed yield, agronomic characteristics and lodging resistance, and to provide a survey with differences between and within species (Table 1).

Buckwheat, corn, lupin, oats, and winter wheat were all grown at the Rosemount Experiment Station. Hull-less waxy barley (HW) and malting barley (cv. Robust) were obtained from Dr. Gary Fulcher, University of Minnesota (St. Paul, MN).

Crude fat analysis. Amaranth seeds were ground for 30 s in a MicroMill model 502 (TechniLab Instruments, Inc., Pequannock, NJ) and were analyzed in triplicate for crude fat by the Soxhlet procedure (29).

TABLE 1
Accessions of *Amaranthus* Analyzed

Species	Accession	Source of accession
A. acutilobus	Ames 13786	Germany
A. albus	Ames 13788	Germany
A. caudatus	PI 490437	Peru
A. cruentus	Ames 1011	United States
	Ames 1964	Benin
	Ames 1973	Nigeria
	Ames 1981	China
	Ames 2049	Indonesia/Java
	Amont 37	Montana, United States
	K283	Pennsylvania, United States
	PI 477914	United States
A. dubius	Ames 13040	Czechoslovakia
A. hybridus	PI 540447	United States
	Ames 5323	Mexico
A. hypochondriacus	K342	Pennsylvania, United States
	PI 540446	Pakistan
	AAI 1492	United States
	Ames 2019A	Puerto Rico
	Ames 2019B	Puerto Rico
	Ames 2030	China
	Ames 2034	Malaysia
	Ames 2265	Pennsylvania, United States
	Ames 5140	United States
A. hypochondriacus × *A. hybridus*	K343 (Plainsman)[a]	Pennsylvania, United States
	K432[a]	Pennsylvania, United States
	K593*a*	Pennsylvania, United States

[a]These lines resulted from selections from *A. hypochondriacus* × *A. hybridus* crosses, breeding work was conducted by Rodale Research Institute (Emmaus, PA).

FAP. The Soxhlet fat extracts of all seeds were analyzed in duplicate for FAP. Residual petroleum ether, boiling range 30–60°C, was removed by gentle boiling immediately after extraction and then in a rotary evaporator with a water bath temperature of 37°C. Methyl ester derivatives were prepared; 200 mg of extracted oil was used instead of the specified 20 mg (30). The fatty acid derivatives were stored at 4°C in amber crimp vials, wrapped in aluminum foil, and were analyzed within a week. Standards of methyl ester derivatives were obtained from Nu-Chek-Prep (Waterville, MN).

The fatty acid derivatives were analyzed with a Hewlett Packard 5890 gas chromatograph (Palo Alto, CA) with flame-ionization detector and Hewlett Packard 7673A automatic injector. The carrier gas was helium, and the samples were injected in split mode with a split ratio of 20:1. The column head pressure was 12 psig. The column was a DB 23 (J&W Scientific, Folsom, CA) with dimensions of 30 m long × 0.32 mm i.d. and 25 μm film thickness. The initial column temperature of 40°C was increased at a rate of 15°C/min to 160°C, where the rate was changed to 5°C/min to a final temperature of 220°C. The length of each run was 27 min.

Tocol analysis. The tocopherols (T) and tocotrienols (T3) were extracted in duplicate from all seeds in minimal light or

complete darkness. One gram of seed was homogenized for one minute in 20 mL of reagent-grade methanol with a Polytron (model 10/35; Brinkmann Instruments, Westbury, NY) on the #4 setting. The samples were then centrifuged (IEC model K; International Equipment Co., Needham Heights, MA) at 5,400 × *g*. The supernatant was removed and placed in a 25-mL glass vial and dried under nitrogen. The pellet was resuspended in 15 mL of reagent-grade methanol, and the homogenization and centrifugation steps were repeated. The supernatant was removed and added to the first extract and dried under nitrogen. The dried extract was resuspended in 2 mL of high-pressure liquid chromatography (HPLC)-grade hexane, mixed briefly in a vortex mixer (model S8220; Scientific Products, McGraw Park, IL), placed in a 2-mL amber crimp vial and immediately analyzed.

The HPLC system (Gilson Medical Electronics Middleton, WI) consisted of a pump (model 302), a manometric module (model 802B), a diluter (model 401), a fluorometer (model 121), and a fraction collector (model 201). Excitation and emission wavelengths were 280 and 240–410 nm, respectively, rather than 290 and 330 nm, respectively, as suggested by Pocklington and Dieffenbacher (31). The column was a LiChrosorb Si 60 (Anspec, Ann Arbor, MI), 250 mm long × 4.6 mm i.d. and 5 μm particle size. The column was washed and conditioned for about 10 min with HPLC-grade methanol (Sigma Chemical Co., St. Louis, MO), then 10 min with dichloromethane (Sigma Chemical Co.), followed by hexane (Fischer Scientific Co., Chicago, IL) at a flow rate of about 1 mL/min. After all analyses were completed, the column was stored in HPLC-grade methanol.

The method used for HPLC analysis of tocols (31) was an isocratic system, consisting of a mobile phase of 99.5% hexane and 0.5% isopropanol (Sigma Chemical Co.) at a flow rate of 1.0 mL/min. The external standards used were the same as in our previous investigation (32). A typical run would last 35 min.

β-Glucan measurements. Thirteen accessions represented in the total fat, fatty acid, and vitamin E analysis were not analyzed for β-glucan contents, due to a lack of adequate sample quantity and were replaced by accessions K283, PI 477914, K432, and K593. A few grams each of 12 accessions of amaranth were dried overnight in a convection oven at 80°C and then stored in a desiccator. The seeds (three millings per sample) were milled in a Retsch mill (model ZM-1; Brinkmann Instruments) with a 0.5-mm sieve, combined and mixed well to assure homogeneity. Barley and rye samples, known to contain 4.7 and 2.1% β-glucans, respectively, served as reference standards to confirm accuracy.

The milled samples were analyzed in duplicate for β-glucan contents (33) the next day. All water used was distilled. The glucose test kit (5X98808) and lichenase/β-glucosidase β-glucan assay kit (5X98805) were purchased from Quest Biocon (Sarasota, FL). The centrifuge was a Heraeus Sepatech Biofuge 15 (Heraeus Sepatech GmbH, Osterode/Harz, Germany). Sample absorbance was read in a Bausch and Lomb Spectronic 20 spectrophotometer (Bausch and Lomb, Rochester, NY) at 505 nm with results reported as % β-glucan on a dry basis.

TIA. Seed samples from 20 accessions of amaranth were each ground to a fine powder for 1 min in a MicroMill model 502. In triplicate, 0.5 g of each was suspended in 50 mL distilled water and shaken for 30 min in a G10 gyratory mechanical shaker (New Brunswick Scientific, New Brunswick, NJ) at 200 rpm. The assay for TIA by Liu and Markakis (34) was used. Benzoyl-DL-arginine-*p*-nitroanilide hydrochloride, Tris buffer (preset crystals), and porcine trypsin were obtained from Sigma Chemical Co. Soybeans purchased from a local healthfood store were used as a reference standard.

Sample absorbance was read in a Bausch and Lomb Spectronic 20 spectrophotometer at 410 nm. TIA is defined as an A_{410} increase of 0.01 under conditions of the assay. TIA is expressed as trypsin units inhibited (TUI) per mg of dry sample.

Statistical analysis. Analysis of variance was performed with the Statistix computer program (version 3.5; Analytical Software, St. Paul, MN) and the General Linear Models procedure. Tukey's Honestly Significant Differences test was used to compare treatment means.

RESULTS AND DISCUSSION

Crude fat. The crude fat content of 21 accessions of amaranth (Table 2) ranged from 5.2 to 7.7% of dry matter. These data indicate some significant variation in oil content among accessions. Ames 13786 was the lowest in crude fat, with Ames 1981 the highest. These results are consistent with previous

TABLE 2
Percent Crude Fat [dry basis (DB)] of 21 Accessions of Amaranth[a]

Accession	% Crude fat (DB)	Standard deviation	Species
Ames 13786	5.2	0.2	*Amaranthus acutilobus*
Ames 13788	5.9	0.4	*A. albus*
PI 490437	6.7	0.1	*A. caudatus*
Ames1011	6.9	0.2	*A. cruentus*
Ames 1964	6.5	0.2	*A. cruentus*
Ames 1973	7.5	0.1	*A. cruentus*
Ames 1981	7.7	0.1	*A. cruentus*
Ames 2049	7.0	0.0	*A. cruentus*
Amont 37	7.0	0.1	*A. cruentus*
Ames 13040	7.6	0.1	*A. dubius*
PI 540447	5.9	0.1	*A. hybridus*
Ames 5323	5.5	0.2	*A. hybridus*
PI 540446	6.0	0.0	*A. hypochondriacus*
AAI 1492	6.2	0.4	*A. hypochondriacus*
Ames 2019A	6.6	0.1	*A. hypochondriacus*
Ames 2019B	6.0	0.3	*A. hypochondriacus*
Ames 2030	5.5	0.8	*A. hypochondriacus*
Ames 2034	5.8	0.1	*A. hypochondriacus*
Ames 2265	6.1	0.1	*A. hypochondriacus*
Ames 5140	6.8	0.2	*A. hypochondriacus*
K343 (Plainsman)	7.2	0.0	*A. hypochondriacus* × *A. hybridus*
Overall mean	6.5	0.2	
HSD ($P \leq 0.05$)	1.0		

[a]Means of triplicate determinations; HSD, Honestly Significant Differences.

TABLE 3
Fatty Acid Profiles of 21 Amaranth Accessions[a]

	Fatty acid (%)							
Accession	Palmitic 16:0	Stearic 18:0	Oleic 18:1	Linoleic 18:2	Linolenic 18:3	All others	S/U ratio[b]	Species
Ames 13786	16.5	3.1	19.0	44.0	0.4	17.0	0.31	*Amaranthus acutilobus*
Ames 13788	7.8	0.7	19.7	60.9	0.0	10.9	0.11	*A. albus*
PI 490437	18.3	3.1	28.0	35.6	0.3	14.7	0.34	*A. caudatus*
Ames 1011	20.1	3.3	27.5	43.0	0.0	6.1	0.33	*A. cruentus*
Ames 1964	17.8	3.5	21.0	41.0	0.0	16.7	0.34	*A. cruentus*
Ames 1973	17.8	3.5	22.5	40.7	0.0	15.5	0.34	*A. cruentus*
Ames 1981	17.8	3.8	22.7	42.4	0.3	13.0	0.33	*A. cruentus*
Ames 2049	15.8	3.6	20.9	38.3	0.7	20.7	0.32	*A. cruentus*
Amont 37	18.3	3.2	28.3	37.0	0.5	12.7	0.33	*A. cruentus*
Ames 13040	16.9	3.5	20.4	46.9	0.4	11.9	0.30	*A. dubius*
PI 540447	19.0	3.6	23.5	44.9	0.0	9.0	0.33	*A. hybridus*
Ames 5323	20.5	2.8	20.8	46.4	0.0	9.5	0.35	*A. hybridus*
PI 540446	20.3	3.1	19.1	49.4	0.0	8.1	0.34	*A. hypochondriacus*
AAI 1492	21.6	3.5	29.8	39.3	0.0	5.8	0.36	*A. hypochondriacus*
Ames 2019A	19.3	2.8	19.1	46.6	0.0	12.2	0.34	*A. hypochondriacus*
Ames 2019B	21.1	3.4	17.5	52.2	0.0	5.8	0.35	*A. hypochondriacus*
Ames 2030	19.7	2.9	20.9	45.6	0.0	10.9	0.34	*A. hypochondriacus*
Ames 2034	21.8	3.0	16.3	52.5	0.0	6.4	0.36	*A. hypochondriacus*
Ames 2265	20.5	3.3	20.5	50.6	0.0	5.1	0.34	*A. hypochondriacus*
Ames 5140	17.9	3.4	20.5	41.1	0.3	16.8	0.34	*A. hypochondriacus*
K343 (Plainsman)	19.1	3.2	24.7	43.0	0.5	9.5	0.33	*A. hypochondriacus* × *A. hybridus*
Overall mean	18.5	3.2	22.0	44.8	0.2	11.3	0.33	
HSD ($P \leq 0.05$)	5.0	1.1	4.0	7.5	NSD	—	—	

[a]Means of duplicate determinations; NSD, no significant difference.
[b]S/U ratio = saturated/unsaturated = (16:0 + 18:0)/(18:1 + 18:2 + 18:3); see Table 2 for other abbreviation.

findings that reported a crude fat range of 4.9 to 8.1% (1). The overall mean crude fat contents of the eight species of amaranth ranged from 5.2 (*A. acutilobus*) to 7.6% (*A. dubius*) of dry matter. Although mean fat content varied significantly ($P \leq 0.05$) among accessions and species in this study, some species were better represented than others. Overall mean fat content across accessions was 6.5 ± 0.2%. This compares well with a total lipid range of 6.5 (*A. hybridus)* to 8.1% (*A. hypochondriacus* × *A. hybridus*) in a study involving eight accessions and four species (35).

FAP. The FAP of extracted amaranth oil (Table 3) showed significant variation ($P \leq 0.05$) among some accessions in the contents of palmitic acid (range, 7.8 to 21.8%), stearic acid (0.7 to 3.8%), oleic acid (16.3 to 29.8%), and linoleic acid (35.6 to 60.9%). Linolenic acid concentrations were not different, but ranged only up to 0.7%. Overall means across accessions in order of decreasing concentration were linoleic, 44.8%; oleic, 22.0%; palmitic, 18.5%; stearic, 3.2%; linolenic, 0.2%; and all others accounting for the remaining 11.3%. The results, in general, are consistent with previous published reports for palmitic acid (12 to 25%), oleic acid (19 to 35%), and linoleic acid (37 to 62%) (1,8,9,21,35). However, three accessions were lower, in palmitic acid (Ames 13788, 7.8%), oleic acid (Ames 2034, 16.3%), and linoleic acid (PI 490437, 35.6%) than previously reported.

The S/U ratios of the oils ranged from 0.31 to 0.36 in 20 of the 21 accessions (Table 3); however, Ames 13788 oil was much more highly unsaturated than the others with a S/U ratio of only 0.11. It appears that there is a greater potential for plant breeders to manipulate fatty acid composition, particularly the 16:0, 18:1, and 18:2 acids, than to substantially alter the total fat content.

Significant variation ($P \leq 0.05$) also occurred among species in the contents of palmitic acid (range, 7.8 to 20.3%), stearic acid (0.7 to 3.5%), oleic acid (19.0 to 28.0%), and linoleic acid (35.6 to 60.9%).

The overall mean FAP and S/U ratios for the amaranth oil are compared in Table 4 with those for oils from HW barley, normal barley (cv. Robust), buckwheat, corn, lupin, oats, and wheat. As reported by Lyon and Becker (8), amaranth oil is similar to corn and cottonseed oils in its fatty acid composition; however, it also appears to be similar to buckwheat oil. Amaranth oil was highest in S/U ratio among those compared in Table 5. Lupin oil (lowest in palmitic, highest in oleic and linoleic) and corn oil (next lowest in palmitic, highest in linoleic) exhibited the lowest S/U ratios.

Vitamin E. The vitamin E profiles of the methanol extracts of amaranth (Table 5) showed significant variation ($P \leq 0.05$) among accessions in the content of αT (range, 0.59 to 2.95 mg/100 g seed), βT (1.01 to 6.74), βT3/γT (0.06 to 0.68), and δT (0.11 to 2.05). It was not possible to separate βT3 and γT in this study. The total tocols ranged from 2.81 (AAI 1492) to 7.83 (Ames 13788) mg/100 g seed. The contents of αT3 and γT3 were low or nonexistent and therefore did not vary

TABLE 4
Overall Mean Fatty Acid Profile of 21 Accessions of Amaranth Compared with Means of Seven Other Grains[a]

	Fatty acid (%)						
Crop	Palmitic 16:0	Stearic 18:0	Oleic 18:1	Linoleic 18:2	Linolenic 18:3	All others	S/U ratio[b]
Amaranth	18.5	3.2	22.0	44.8	0.2	11.3	0.33
Barley HW	21.0	0.0	15.6	52.4	5.6	5.4	0.29
Barley Robust	18.5	0.0	15.2	54.0	5.5	6.8	0.25
Buckwheat	18.2	0.0	36.4	34.8	0.0	10.6	0.26
Corn	10.3	0.6	21.4	62.9	0.5	4.3	0.13
Lupin	9.0	0.0	57.5	16.7	10.9	5.9	0.11
Oats	17.3	0.0	39.8	38.5	0.0	4.4	0.22
Wheat	15.4	0.0	22.3	54.2	3.5	4.6	0.19

[a]Means of duplicate determinations.
[b]S/U ratio = saturated/unsaturated = (16:0 + 18:0)/(18:1 + 18:2 + 18:3).

among accessions. None of the accessions contained measurable quantities of δT3.

Similarly, significant differences were found among species in αT (range, 0.59 to 2.14 mg/100 g seed), αT3 (0.00 to 0.11), βT (1.31 to 6.74), and δT (0.14 to 1.58). However, the βT3/γT, γT3, and δT3 contents were low or nonexistent and therefore not significantly different among species. Among species, total tocols ranged from 3.46 (*A. acutilobus*) to 7.83 (*A. albus*) mg/100 g seed. As was noted for the fat contents and FAP, some species were better represented than others in this study.

The data in Table 6 suggest that amaranth is not unique in vitamin E content relative to other grains. It contained a substantially lower quantity of total tocols than corn and lupin and only small or negligible amounts of αT3, βT3, γT3. None of the others contained detectable amounts of δT3. The tocols of the non-amaranth seeds are in qualitative agreement with previous investigations (36). The results, in general, are in sharp contrast, both quantitatively and qualitatively, with a previous study (11), which reported relatively high levels of βT3 and γT3 but no βT in grain amaranth.

Quantitative differences in composition can occur, due to variations in analytical methodology and technique as well as in the conditions of growing, harvesting, and storing the crop (28). However, the qualitative differences between these present data and those of Lehmann *et al.* (11) are greater than one

TABLE 5
Vitamin E Profiles [wet basis (WB)] of 21 Amaranth Accessions[a]

	Tocol (mg/100 g seed; WB)								
Accession	αT	αT3	βT	βT3/γT	γT3	δT	δT3	Total	Species
Ames 13786	1.21	0.10	1.82	0.17	0.00	0.16	0.00	3.46	*Amaranthus acutilobus*
Ames 13788	0.80	0.09	6.74	0.06	0.00	0.14	0.00	7.83	*A. albus*
PI 490437	1.47	0.09	1.65	0.16	0.00	0.37	0.00	3.74	*A. caudatus*
Ames 1011	1.87	0.00	1.90	0.40	0.00	0.77	0.00	4.94	*A. cruentus*
Ames 1964	2.07	0.00	1.07	0.20	0.00	0.16	0.00	3.50	*A. cruentus*
Ames 1973	1.81	0.04	1.09	0.19	0.00	0.13	0.00	3.26	*A. cruentus*
Ames 1981	2.29	0.00	1.74	0.23	0.00	0.31	0.00	4.57	*A. cruentus*
Ames 2049	1.85	0.04	1.01	0.14	0.00	0.11	0.00	3.15	*A. cruentus*
Amont 37	2.95	0.05	2.14	0.17	0.00	0.41	0.03	5.75	*A. cruentus*
Ames 13040	0.59	0.10	3.14	0.22	0.00	0.20	0.00	4.25	*A. dubius*
PI 540447	1.66	0.00	2.58	0.28	0.00	1.44	0.03	5.99	*A. hybridus*
Ames 5323	1.65	0.08	2.90	0.34	0.00	1.72	0.00	6.69	*A. hybridus*
PI 540446	1.58	0.08	2.68	0.46	0.06	2.05	0.00	6.91	*A. hypochondriacus*
AAI 1492	0.78	0.00	1.61	0.17	0.00	0.25	0.00	2.81	*A. hypochondriacus*
Ames 2019A	2.10	0.04	1.79	0.51	0.00	1.70	0.00	6.14	*A. hypochondriacus*
Ames 2019B	1.60	0.00	2.46	0.22	0.00	0.69	0.00	4.97	*A. hypochondriacus*
Ames 2030	2.11	0.00	2.00	0.51	0.00	1.37	0.03	6.02	*A. hypochondriacus*
Ames 2034	1.45	0.00	2.56	0.31	0.00	1.71	0.00	6.03	*A. hypochondriacus*
Ames 2265	1.39	0.11	1.50	0.68	0.00	1.17	0.00	4.85	*A. hypochondriacus*
Ames 5140	1.56	0.00	1.97	0.19	0.00	0.47	0.00	4.19	*A. hypochondriacus*
K343 (Plainsman)	2.02	0.00	1.31	0.39	0.00	0.99	0.00	4.71	*A. hypochondriacus* × *A. hybridus*
Overall mean	1.66	0.04	2.17	0.29	0.00	0.78	0.00	4.94	
HSD ($P \leq 0.05$)	0.83	NSD	0.71	0.39	NSD	0.48	NSD	—	

[a]Means of duplicate determinations; T, tocopherol; T3, tocotrienols; see Tables 2 and 3 for other abbreviations.

TABLE 6
Overall Mean Vitamin E Profiles of 21 Accessions of Amaranth Compared with Means of Seven Other Grains[a]

	Tocol (mg/100 g seed; WB)							
Crop	αT	αT3	βT	βT3/γT	γT3	δT	δT3	Total
Amaranth	1.66	0.04	2.17	0.29	0.00	0.78	0.00	4.94
Barley HW	0.89	0.85	0.18	0.35	0.46	0.07	0.00	2.80
Barley Robust	0.93	0.67	0.14	0.36	0.35	0.03	0.00	2.48
Buckwheat	0.46	0.04	0.00	2.89	0.00	0.15	0.00	3.54
Corn	0.89	0.24	0.18	2.85	0.47	0.10	0.00	4.73
Lupin	0.62	0.05	0.25	6.12	0.17	0.18	0.00	7.39
Oats	1.00	0.54	0.15	0.42	0.00	0.03	0.00	2.14
Wheat	1.13	0.12	0.43	6.20	0.00	0.00	0.00	7.88

[a]Means of duplicate determinations; WB, wet basis. See Figure 5 for other abbreviations.

might expect when considering qualitative variation in vitamin E profiles reported by several investigators for other crop seeds (36). Because this study found no appreciable amounts of tocotrienols in any of the amaranth accessions, it is more likely that substances other than tocotrienols were responsible for reported cholesterol-lowering effects of grain amaranth in rats (14,15) and chicks (16) as other investigators have suggested (13,17).

β-Glucan content. No reports were found in the literature of the β-glucan content of amaranth. Because β-glucans have been documented as being hypocholesterolemic agents, twelve accessions of amaranth representing the important agronomic species in North America were analyzed for their contents of (1 → 3), (1 → 4) β-glucans (Table 7). None of the twelve contained these β-glucans at more than 0.5%, thus minimizing the role of these specific compounds as the hypocholesterolemic agents in rat (14,15) and chick (16) feeding studies. Apparently, the active agent and its mode of action are yet to be identified.

TIA. The literature has reported varying levels of TIA in amaranth grain, usually of a specific individual species. To assess TIA over a broad range of germplasm, grain from twenty accessions, representing six amaranth species, was analyzed. The TUI (Table 8) ranged up to only 4.3 TUI/mg compared to 110.7 TUI/mg for the raw soybean reference sample. Because these levels fall well below those known to adversely affect weight gains in rats (37), statistical analysis was not undertaken. The results indicate that, although some TIA apparently exists in this broad range of amaranth accessions, it is at low levels and well within the recognized margin of safety.

In conclusion, the total fat content in 21 accessions (eight species) of amaranth adaptable to the upper Midwest varied over a rather narrow range (5.2 to 7.7%). A wider range of

TABLE 7
Percent β-Glucan [(dry basis (DB)] of 12 Accessions of Amaranth[a]

Accession	% β-Glucan (DB)	Species
Ames 1964	0.3	*Amaranthus cruentus*
Ames 1973	0.3	*A. cruentus*
Ames 2049	0.1	*A. cruentus*
Amont 37	0.1	*A. cruentus*
K283	0.4	*A. cruentus*
PI 540447	0.5	*A. hybridus*
Ames 5323	0.4	*A. hybridus*
PI 477914	0.4	*A. hypochondriacus*
Ames 2034	0.4	*A. hypochondriacus*
K343 (Plainsman)	0.5	*A. hypochondriacus* × *A. hybridus*
K432	0.4	*A. hypochondriacus* × *A. hybridus*
K593	0.5	*A. hypochondriacus* × *A. hybridus*
Overall mean	0.4	
Rye-reference	2.1	
Barley-reference	4.7	

[a]Means of duplicate determinations.

TABLE 8
Trypsin Inhibitor Activity [(wet basis (WB)] of 20 Accessions of Amaranth[a]

Accession	TUI/mg (WB)	Species
Ames 13788	0.9	*Amaranthus albus*
Ames 1964	3.1	*A. cruentus*
Ames 1973	1.6	*A. cruentus*
Ames 1981	3.3	*A. cruentus*
Ames 2049	2.7	*A. cruentus*
K283	0.0	*A. cruentus*
Ames 13040	0.1	*A. dubius*
PI 540447	0.0	*A. hybridus*
Ames 5323	0.0	*A. hybridus*
K342	1.1	*A. hypochondriacus*
PI 477914	4.3	*A. hypochondriacus*
PI 540446	0.0	*A. hypochondriacus*
AAI 1492	3.0	*A. hypochondriacus*
Ames 2019A	0.0	*A. hypochondriacus*
Ames 2019B	0.1	*A. hypochondriacus*
Ames 2030	0.0	*A. hypochondriacus*
Ames 2034	0.1	*A. hypochondriacus*
Ames 2265	0.0	*A. hypochondriacus*
Ames 5140	0.2	*A. hypochondriacus*
K343 (Plainsman)	1.1	*A. hypochondriacus* × *A. hybridus*
K593	1.2	*A. hypochondriacus* × *A. hybridus*
Overall mean	1.1	
Raw soybean	110.7	

[a]Means of two true replicates each analyzed twice.

variation was observed in the component fatty acid contents, suggesting a greater potential for plant breeders to manipulate fatty acid composition, including S/U ratio, than total fat content. Vitamin E contents were comparable to those of other grain crops, and tocotrienols were essentially absent. This finding, along with the virtual absence of $(1 \rightarrow 3)$, $(1 \rightarrow 4)$ β-glucans, indicates that any hypocholesterolemic activity of amaranth must be attributed to some other as yet unidentified constituent(s). TIA, where it existed at all, was at low enough levels so as not to present a health concern.

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