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Assessment of the protein quality of twenty nine grain amaranth (*Amaranthus* spp. L.) accessions using amino acid analysis and one-dimensional electrophoresis

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Protein deficiency in diets adversely affects growth and development. Novel source of high quality protein and its utilization is essential in improving the nutritive status of the vulnerable groups. Total protein content and protein fractions of 29 amaranth accessions and a soybean cultivar used as reference were determined. The amino acid composition of ten representative accessions of amaranth was also determined. Total protein content ranged from 11.77 to 19.01 g/100 g. One-dimensional gel electrophoretic separations revealed albumin, globulin and glutelin as the major protein fractions; prolamin was not detected. All accessions had similar seed protein electrophoretic profile, ranging from 6.5 to 66 kDa. The glutelin fraction of all amaranth accessions shared similar electrophoretic bands with the soybean cultivar at the 4 to 14, 24 to 36 and 65 to 66 kDa regions. All amaranth accessions contained a good balance of the nine essential amino acids. The sum of essential amino acids ranged from 31.22 to 44.88 g/100 g and 60.87 g/100 g total protein in amaranth and soybean, respectively; limited only in tryptophan and leucine for amaranth, and methionine for soybean. Amaranth is a good source of high quality protein and may serve as a nutritive substitute for some cereals in functional foods.

Key words: Amaranthus, amino acid, gel electrophoresis, protein quality, protein fractions.

INTRODUCTION

Proteins constitute an important group of biomacromolecules that are involved in physiological functions (Wright, 1987). Protein malnutrition is still a major challenge in developing countries (UNICEF, 2009; Black et al., 2008). Therefore, it is essential to broaden the food base by utilization of underutilized crops with promising nutritive potential as recognized by National Academy of Science (NAS) (1984). Natural vegetable proteins are useful materials owing to their safeness, high biocompatibility, nutritional value and low cost. Finding new vegetable proteins rich in essential amino acids is important for the food and pharmaceutical industries (Paredes-Lopez et al., 1988). Most cereals are deficient in some amino acids, for instance, corn is deficient in

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lysine and tryptophan, rice in lysine and threonine (Deshpande et al., 1955; Bressani and Garcia-Vela, 1990) and wheat in lysine (Howe et al., 1965). Amaranth (*Amaranthus*) belongs to a nutritious class of pseudo-cereals and it has been identified as a very promising food crop because of its exceptional nutritive value as judged by its protein and lipid content, as well as for its essential amino acid composition that has relatively high lysine content (Teutonico and Knorr, 1985). There are three main species of amaranth with desirable agronomic traits namely, *Amaranthus caudatus, Amaranthus cruentus* and *Amaranthus hypochondriacus*. The protein content of *A. cruentus, A. caudatus* and *A. hypochondriacus* are 13.2 to 18.2 g/100 g, 17.6 to 18.4 g/100 g and 17.9 g/100 g, respectively (Gorinstein et al., 1998).

The lysine contents of amaranth species are high ranging from 3.2 to 6.4 g/100 g when compared with those found in most cereals, 2.2 to 4.5 g/100 g. The sulphur containing amino acids (2.6 to 5.5 g/100 g) is higher than that of the most important legumes (1.4 g/100 g) such as pea, beans and soybeans (Gorinstein et al., 1998; Juan et al., 2007). In addition, amaranths are also good sources of minerals and vitamins and they contain larger amounts of these nutrients than most of the common cereals and legumes (Muyonga et al., 2008).

The major storage proteins in most cereal grains such as wheat, barley, rye, maize and sorghum are the alcohol-water soluble prolamins (Gorinstein et al., 1991a; Kreis et al., 1985). Oats and rice have globulins and glutelins, respectively, as major proteins but small amounts of prolamins (Yamagata et al., 1982). In amaranth, the major storage proteins are the globulins and glutelins, while the amount of alcohol-soluble prolamins is low as in oats and rice (Gorinstein et al., 1991a).

Protein content and amino acid composition depend on genotype and growing conditions (Gorinstein et al., 2002). Also, the storage protein composition of soybean has been reported to be influenced by plant nutrient availability (Krishnan et al., 2005). Most investigations on amaranth species have focused on storage protein fractions. However, assessment of the protein quality of *Amaranthus* species based on total protein content, protein fractions and amino acid composition, with reference to soybean an excellent source of high-quality protein (Zarkadas et al., 2007b) and amino acid requirement for human according to the FAO/WHO (1991) and the USFDA (1993) compositional data is scarce.

Thus, this study was carried out to determine the levels of total protein and individual amino acids in 29 grain amaranth accessions grown under field conditions at the National Horticultural Research Institute (NIHORT), Ibadan, Nigeria. Amaranth accessions were also screened for differences in genetic variability in storage protein subunits by 1-DE gel electrophoresis and were compared with the soybean cultivar used as reference.

Findings from this study would assist plant breeders and nutritionists in their selection of high protein quality amaranth accessions.

MATERIALS AND METHODS

Twenty nine accessions of *Amaranthus* belonging to five species: *A. caudatus, A. cruentus, Amaranthus hybrid, A. hypochondriacus* and *Amaranthus hybridus* were used in this study (Table 1). Twenty seven of the accessions were obtained from the USDA-ARS North Central Regional Plant Introduction Station (NCRPIS) in Ames, USA; and two were obtained from the National Horticultural Research Institute (NIHORT) germplasm, Ibadan, Nigeria. The twenty nine accessions were planted in the experimental field of NIHORT in 2010 in three replicates in a randomized complete block design and harvested at maturity.

Sample preparation

Whole mature seeds of each accession planted in 3 replicates were pooled together and ground in a mill. The resulting flour was defatted with n-hexane at a flour/hexane ratio of 1:10 (w/v) prior to protein extraction and fractionation (Barba de la Rosa et al., 1992).

Protein extraction

Proteins were extracted stepwise according to solubility in different solvents following the method of Landry and Moureaux (1980). Fractionation sequences were performed first in distilled water (albumin), followed by 0.5 M NaCl (globulin), 55% 2-propanol (prolamin-like), 55% 2-propanol with 0.6% 2-mercaptoethanol (prolamin), sodium borate buffer (pH 10) with 0.6% 2-mercaptoethanol and 0.5 M NaCl (glutelin-like), borate buffer (pH 10) with 0.6% 2-mercaptoethanol and 0.5% sodium dodecyl sulfate (glutelin).

Protein determination

Nitrogen content was determined by the micro-Kjeldahl method (Hach, 1990) and the protein content was calculated using the nitrogen/protein conversion factor of 6.25.

One dimensional electrophoresis (1-DE)

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the procedure of Laemmli (1970). Protein extracts were combined and diluted in a sample buffer that contained 60 mM Tris-HCl (pH 6.8), 2% w/v SDS, 3.33% v/v β-mercaptoethanol, 10% glycerol and 0.05% of bromophenol blue. The samples were heated at 98°C for 5 min before loading onto a vertical slab gel (Mini-PROTEAN II Electrophoresis Cell) using a 12% gradient separating (acrylamide/bis acrylamide) gel and a 4% (w/v) stacking gel containing 0.1% SDS. Low range molecular weight marker (6.5 to 66 kDa) obtained from Sigma Chemical Co was used for the estimation of the protein subunits. Molecular weights standard are: bovine serum albumin (66 kDa), ovalbumin (45 kDa), phosphate dehydrogenase (36 kDa), trypsinogen (24 kDa), trypsin inhibitor (20 kDa) and aprotinin (6.5 kDa). Electrophoresis was done at 125 V for about 2 h. At the end of the run, the gels were stained with Coomasie Brilliant Blue R250 in methanol/water/acetic acid (4:5:1 v/v/v) and destained with methanol/water/acetic acid (4:5:1 v/v/v) over night.

Table 1. Species and accessions of 29 grain amaranth used in this study, their pedigree and total protein contents (g/100 g).

Species	Accession Number[a]	Accession code	Origin	Plant name	Total protein content (g/100 g)
Amaranthus caudatus	1	P1 490458	Bolivia	LSK 38	14.12±0.26
	2	PI 511679	Argentina	RRC 551	17.30±1.12
	3	PI 553073	United States, New Jersey	LOVE-LIES-BLESSING	11.77±0.26
	4	P1 642741	Bolivia	Oscar Blanco	13.83±0.15
Amaranthus cruentus	5	PI 477913	Mexico	RRC 1011	16.10±0.20
	6	PI 511719	Guatemala	Niqua, alegria, chang	15.04±0.59
	7	PI 515959	United states, Montana	Montana-3	15.39±0.11
	8	PI 538319	United states, Pennsylvania	K266	16.03±0.09
	9	PI 590992	China	TIBET	18.42±0.17
	10	P1 604666	United states, Pennsylvania	RRC 1027	14.45±0.13
	11	PI 641047	Nigeria, Oyo	CEN/IB/97/AMA008	15.71±0.06
	12	PI 641045	Nigeria, Oyo	CEN/IB/97/AMA005	15.36±0.08
Amaranthus hybrid	13	PI 538325	United states, Pennsylvania	K593	16.57±0.08
	14	PI 538326	United States, Pennsylvania	D70-1	13.63±0.09
	15	PI 538327	United States, Pennsylvania	DI36-1	17.75±0.09
	16	Ames 1974	Nigeria	RRC 18C	16.46±0.08
	17	Ames 2256	Nigeria	SP 12C	17.96±0.62
	18	Ames 5644	Nigeria	RRC 1044	12.81±0.30
	19	Ames 5647	Nigeria	RRC 1047	15.27±0.82
Amaranthus hypochondriacus	20	PI 337611	Uganda	P 373	13.04±0.20
	21	PI 511731	Mexico	RRC 646	12.76±0.28
	22	PI 558499	United States, Nebraska	PLAINSMAN	12.82±0.04
	23	PI 590991	China, Shanxi	ZHEN PING	14.01±0.35
	24	PI 615696	India, Himachal Pradesh	Amapurna	15.16±0.12
	25	P1 619250	United States, Pennsylvania	K 116	14.51±0.51
	26	PI 633596	Nepal	Jumla	12.72±0.56
	27	Ames 1972	Nigeria	RRC 18A	15.53±0.43
Amaranthus hybridus	28	NH 84/444-4	Nigeria	NH Purple	19.01±0.21
	29	NAC 3	Nigeria	NH Green	16.77±0.73

Amino acid analysis

Only 10 out of the 29 amaranth accessions were used for this analysis; and they included two accessions from each of the five amaranth species. A soybean cultivar (TGX 1448-2E) obtained from International Institute of Tropical Agriculture (IITA) germplasm, was also included in this study and used for comparison as a known high quality protein.

Amino acid analysis was carried out at AltaBioscience Laboratory, University of Birmingham, Edgbaston, UK, according to the methods of Spackman et al. (1958) and Barrett (1985). Amino acid score (AAS) is the concentration of the limiting amino acid in

the food protein, which is expressed according to the method of Young and Steinke (1992) and Zarkadas et al. (2007b) as the proportion or percentage of the concentration of the same amino acid in a standard or reference pattern such as for the diet of a 2-5-year-old child.

$$AAS = \frac{\text{AA content (mg/g of protein) of food protein}}{\text{AA content of FAO/WHO (1991) pattern for a 2-5-year old child}}$$

Statistical analysis

Analysis of variance was done on the protein and amino acid data using Statistical Analysis System (SAS) (2003). Duncan multiple range test was used to determine significant differences among the amaranth accessions. P values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The total protein content of the twenty nine amaranth accessions evaluated ranged from 11.77 to 19.01 g/100 g and differed significantly ($p<0.05$) among the accessions (Table 1). *A. hybridus* (accession 28) had the highest protein content (19.01 g/100 g), followed by *A. cruentus* (accession 9) having 18.42 g/100 g and *A. hybrid* (accession 17) having 17.96 g/100 g. The total protein content of most of the amaranth accessions evaluated in this study were higher than values obtained for wheat (13.5 to 14.5 g/100 g), maize (10.6 to 13.8 g/100 g), barley (10 to 14.9 g/100 g) and oats (12.4 to 12.9 g/100 g) by Gorinstein et al. (2002); but lower than 32 to 38 g/100 g obtained for soybean by Zarkadas et al. (2007a, b). Total protein contents (11.77 to 19.01 g/100 g) of the 29 amaranth accessions are similar to 13 to 18 g/100 g obtained for some amaranth species by Dagmar et al. (2012). Bressani and Garcia-Vela (1990) also reported the total protein content of *A. caudatus, A. hypochondriacus and A. cruentus* to be 14.5 to 14.6, 14.7 to 15.9 and 15.3 to 16.1 g/100 g, respectively. Similar results were obtained for the total protein content of *A. caudatus* (16.6 g/100 g) by Gorinstein et al. (1998, 1999), *A. caudatus* (11.0 g/100 g) by Repo-Carrasco-Valencia et al. (2010) and *A. hypochondriacus* (14.2 to 15 g/100 g) by Czerwinski et al. (2004).

Significant variation ($p<0.05$) in the amino acid composition was observed among the ten selected amaranth accessions evaluated (Table 2). The amaranth accessions evaluated were high in glutamic acid, aspartic acid, arginine, glycine and lysine. Similar trend was observed in the amino acid composition of the soybean cultivar (TGX 1448-2E) used for comparison in this study. Zarkadas et al. (2007a, b) reported high levels of glutamic acid, aspartic acid, arginine, lysine, leucine and glycine in some soybean cultivars evaluated. Most of the amaranth accessions evaluated in this study were observed to have higher glycine, methionine and cysteine contents than the soybean cultivar used as reference, while non-essential amino acids were higher in soybean than in amaranth. The lysine contents: 7.89 g/100 g protein of *A. hypochondriacus* (accession 27), 7.88 g/100 g protein of *A. hybridus* (accession 28) and 7.77 g/100 g protein of *A. caudatus* (accession 2) are higher than the lysine contents (7.0 g/100 g protein) obtained in hen's whole egg (FAO/WHO, 1991); 3.2 to 6.4 g/100 g protein obtained in amaranth (Gorinstein et al., 1991b) and 2.2 to 4.5 g/100 g protein found among the most common cereals (Gorinstein et al., 1998). The sulphur containing amino acids (methionine + cysteine) contents of amaranth accessions in this study ranged from 1.77 to 2.74 g/100 g protein with some accessions having higher values than 2.15 g/100 g protein obtained for the soybean cultivar (TGX 1448-2E). The methionine + cysteine contents of the amaranth accessions evaluated were also higher than 1.4 g/100 g protein obtained for important legumes such as pea and beans (Gorinstein et al., 1998). All amaranth accessions evaluated had higher histidine, lysine and threonine contents than values obtained for hen's whole egg (FAO/WHO, 1991).

The essential amino acid EAA profiles and protein ratings of the 10 accessions of amaranth evaluated are compared with reference amino acid patterns for humans (requirement for a 2-5-year old preschool child) as recommended by FAO/WHO (1991) and USFDA (1993) (Table 3). All amaranth accessions evaluated contained all the nine essential amino acids (EAA_9) with values higher than the EAA requirement for a 2-5-year old preschool child. Amaranth accessions were limited only in tryptophan, which ranged from 0.72 to 0.91 g/100 g per protein and is comparable to 1.1 g/100 g per protein recommended for a 2-5-year old preschool child (FAO/WHO, 1991).

The sum of essential amino acids of amaranth in this study ranged from 31.22 to 44.88 g/100 g per protein and is lower than 60.87 g/100 g per protein obtained for the soybean cultivar (TGX 1448-2E) used for comparison. Results of this study are similar to earlier results obtained in grain amaranth (47.6 g/100 g) and soybean (60.3 g/100 g) by Gorinstein et al. (2002). Amaranth and soybean have higher amounts of total essential amino acids than 33.9 g/100 g reference protein pattern value of FAO/WHO (1991) for the diet of a 2- to 5-year-old child, and close to 51.2 g/100 g given for hen's whole egg. These results indicate that grain amaranth provide a good balance of total essential amino acids.

From the calculated amino acid score, the limiting amino acid in amaranth is tryptophan and leucine while in soybean, it is methionine and tryptophan (Table 4). Result of this study is not in agreement with result of Bressani et al. (1987) in which threonine is the limiting amino acid in amaranth protein. Earlier assessment of protein quality of soybean reported methionine as the limiting amino acid (Zarkadas et al., 2007a), this is similar to result of the soybean cultivar (TGX 1448-2E) used as reference in this study.

Table 2. Comparison of the amino acid (AA) composition and total protein content (g/100 g of total protein; mean ± SD) of ten selected grain amaranth accessions with a soybean cultivar (TGX 1448-2E).

AA	2	3	9	10	17	18	21	23	27	28	Soybean (TGX 1448-2E)	Mean AA	LSD	CV
Aspartic acid	11.20 ± 0.20	8.60 ± 0.60	10.60 ± 0.52	10.80 ± 0.60	10.80 ± 0.36	9.47 ± 0.10	8.87 ± 0.10	11.40 ± 0.20	11.50 ± 0.40	11.00 ± 0.50	20.57 ± 0.45	13.22	0.69	3.09
Threonine	4.82 ± 0.02	3.50 ± 0.44	4.53 ± 0.03	4.58 ± 0.03	4.50 ± 0.30	4.22 ± 0.04	3.86 ± 0.06	4.78 ± 0.03	5.11 ± 0.03	4.79 ± 0.03	6.70 ± 0.30	5.28	0.31	3.52
Serine	7.37 ± 0.02	5.79 ± 0.02	7.11 ± 0.02	7.39 ± 0.10	7.22 ± 0.20	6.20 ± 0.20	5.70 ± 0.60	7.61 ± 0.01	8.14 ± 0.04	7.48 ± 0.06	8.40 ± 0.26	7.89	0.37	2.77
Glutamic acid	22.90 ± 0.40	14.10 ± 0.17	19.90 ± 0.90	22.40 ± 0.20	21.70 ± 0.30	18.40 ± 0.40	16.00 ± 0.57	23.20 ± 0.10	23.70 ± 0.20	21.50 ± 0.50	29.10 ± 0.26	23.82	0.72	1.78
Proline	4.95 ± 0.03	3.42 ± 0.02	4.64 ± 0.04	5.02 ± 0.03	5.05 ± 0.02	4.14 ± 0.03	3.39 ± 0.02	5.22 ± 0.11	5.23 ± 0.10	4.87 ± 0.07	7.60 ± 0.20	5.56	0.14	1.46
Glycine	8.82 ± 0.02	6.96 ± 0.17	8.03 ± 0.03	8.81 ± 0.01	8.67 ± 0.07	7.44 ± 0.04	6.97 ± 0.02	9.11 ± 0.10	9.04 ± 0.03	8.24 ± 0.02	6.10 ± 0.00	8.58	0.11	0.79
Alanine	4.68 ± 0.08	3.30 ± 0.30	4.30 ± 0.20	4.34 ± 0.01	4.37 ± 0.04	4.06 ± 0.03	3.59 ± 0.20	4.71 ± 0.01	4.70 ± 0.03	4.54 ± 0.04	6.45 ± 0.30	5.04	0.27	3.11
Cysteine	2.00 ± 0.20	1.13 ± 0.03	1.57 ± 0.02	1.88 ± 0.08	1.57 ± 0.03	1.32 ± 0.02	0.98 ± 0.01	1.54 ± 0.04	1.55 ± 0.03	1.47 ± 0.03	1.50 ± 0.07	1.64	0.12	4.41
Valine	5.15 ± 0.04	3.64 ± 0.03	4.69 ± 0.06	4.86 ± 0.05	4.98 ± 0.03	4.61 ± 0.01	4.19 ± 0.01	5.35 ± 0.05	5.38 ± 0.05	5.16 ± 0.02	7.30 ± 0.60	5.69	0.31	3.24
Methionine	2.74 ± 0.04	1.80 ± 0.20	2.35 ± 0.04	2.44 ± 0.04	2.32 ± 0.02	2.49 ± 0.02	1.77 ± 0.10	2.56 ± 0.10	2.45 ± 0.04	2.45 ± 0.02	2.15 ± 0.20	2.52	0.17	3.93
Isoleucine	4.75 ± 0.05	3.31 ± 0.02	4.37 ± 0.02	4.48 ± 0.02	4.65 ± 0.04	4.19 ± 0.02	3.94 ± 0.02	4.89 ± 0.02	4.98 ± 0.03	4.75 ± 0.10	7.25 ± 0.50	5.35	0.26	2.92
Leucine	7.53 ± 0.03	5.33 ± 0.03	6.94 ± 0.04	7.10 ± 0.01	7.22 ± 0.20	6.55 ± 0.15	6.00 ± 0.87	7.65 ± 0.02	7.74 ± 0.03	7.38 ± 0.03	12.25 ± 0.40	8.54	0.5	3.49
Tyrosine	3.12 ± 0.10	2.12 ± 0.01	2.82 ± 0.02	3.26 ± 0.05	2.88 ± 0.02	2.44 ± 0.03	2.27 ± 0.02	2.92 ± 0.02	3.06 ± 0.06	3.05 ± 0.05	4.95 ± 0.40	3.44	0.22	3.73
Phenylalanine	5.71 ± 0.02	4.04 ± 0.04	5.01 ± 0.01	5.39 ± 0.05	5.31 ± 0.00	4.77 ± 0.10	4.43 ± 0.02	5.66 ± 0.06	5.74 ± 0.04	5.44 ± 0.04	8.20 ± 0.20	6.17	0.13	1.21
Histidine	4.49 ± 0.10	3.07 ± 0.02	4.22 ± 0.10	4.48 ± 0.03	4.45 ± 0.03	3.53 ± 0.03	3.36 ± 0.06	4.53 ± 0.03	4.70 ± 0.20	4.30 ± 0.30	5.29 ± 0.11	4.70	0.21	2.65
Lysine	7.77 ± 0.02	5.65 ± 0.08	7.01 ± 0.01	7.42 ± 0.03	7.30 ± 0.30	6.84 ± 0.03	6.06 ± 0.06	7.88 ± 0.08	7.89 ± 0.07	7.55 ± 0.05	10.45 ± 0.70	8.39	0.40	2.80
Arginine	11.5 ± 0.50	6.70 ± 0.50	9.94 ± 0.03	11.30 ± 0.00	11.20 ± 0.40	9.13 ± 0.02	8.09 ± 0.05	11.40 ± 0.20	11.80 ± 0.70	11.40 ± 0.05	12.15 ± 0.20	11.52	0.57	2.91
Tryptophan	0.91 ± 0.02	0.88 ± 0.04	0.84 ± 0.04	0.91 ± 0.07	0.79 ± 0.04	0.72 ± 0.08	0.78 ± 0.08	0.91 ± 0.03	0.89 ± 0.04	0.81 ± 0.08	1.28 ± 0.26	0.88	0.16	1.09
Total protein (g/100 g dry matter)	17.30	11.77	18.42	14.45	17.96	12.81	12.76	14.01	15.53	19.01	32.63	15.40	0.73	2.65

Mean values and standard deviation (±) for three replicates.

Both amaranth and soybean could supply preschool child and adult requirements of histidine, isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, threonine and valine.

The sodium dodecyl sulfate polyacrylamide gel electrophoretic separation (Figures 1 to 3) revealed that albumin, globulin and glutelin are the major storage proteins in amaranth. Albumins, globulins and glutelins showed similar patterns for homologous protein fractions isolated from different species. Earlier reports of Silva-Sanchez et al. (2008), Czerwinski et al. (2004) and Gorinstein et al. (1998) showed that albumin and globulin are the major fractions of amaranth seed proteins. Prolamin was not detected in all the 29 grain amaranth accessions evaluated, this is not in agreement with results of Gorinstein et al. (1998) who reported the presence of prolamin, though in very low amount which may be due to the fact that it is probably derived from the perisperm of the amaranth seeds. Konishi et al. (1985) and Gorinstein et al. (1991) reported that the amount of alcohol soluble proteins in amaranth was as low as in oats and rice. Albumins are a protein fraction with polypeptides of very heterogenous sizes, with low molecular weight components being the most abundant as observed in the 20 and 24 kDa regions (Figure 1). Electrophoretic pattern of albumin fractions of all the 29 accessions was similar; all subunits migrated between 6.5 and 66 kDa and had characteristic bands at 20 and 24 kDa, this was similar to the soybean cultivar used for comparison. Accessions 5, 6, 7 (*A. cruentus*) and 14 and 15 (*A. hybrid*) differed from others mainly in the region of 22 kDa. Globulin fractions had distinct bands at 14 to 36 kDa and just above the 45 kDa and shared similar bands with soybean cultivar used for reference in the regions of 20 to 36 kDa and just above the 45 kDa (Figure 2). Result of this study is similar to result of

Table 3. Comparison of the essential amino acid (EAA) scores of ten selected grain amaranth accessions and one soybean cultivar with hen's whole egg, and the FAO/WHO EAA Requirements of a 2-5-year-old child. The values are in mg of amino acid/g of total protein.

EAA	EAA[a] requirements for a preschool child (2-5 year old)	2[b]	3	9	10	17	18	21	23	27	28	Soybean (TGX 1448-2E)	Egg[a]
Histidine	19	44.9	30.7	42.2	44.8	44.5	35.3	33.6	45.3	47.0	43.0	52.9	22
Isoleucine	28	47.5	33.1	43.7	44.8	46.5	41.9	39.4	48.9	49.8	47.5	72.5	54
Leucine	66	75.3	53.3	69.4	71.0	72.2	65.5	60.0	76.5	77.4	73.8	122.5	86
Lysine	58	77.7	56.5	70.1	74.2	73.0	68.4	60.6	78.8	78.9	75.5	104.5	70
Methionine + cysteine	25	47.4	29.3	39.2	43.2	38.9	38.1	18.68	41.0	40.0	39.2	36.5	57
Phenylalanine + tyrosine	63	88.3	61.6	78.3	86.5	81.9	72.1	67.0	85.8	88.0	84.9	131.5	93
Threonine	34	48.2	35.0	45.3	45.8	45.0	42.2	38.6	47.8	51.1	47.9	67.0	47
Tryptophan	11	9.1	8.8	8.4	9.07	7.9	7.2	7.8	9.1	8.9	8.07	12.8	17
Valine	35	51.5	36.4	46.9	48.6	49.8	46.1	41.9	53.5	53.8	51.6	73.0	66
mg/g Total protein EAA9	339	438.7	312.2	399.6	416.6	415.2	379.2	343.9	442.1	448.8	426.3	608.7	512
Total protein (%) EAA9	33.9	43.9	31.2	40.0	41.7	41.5	37.9	34.4	44.2	44.9	42.6	60.9	51.2
Percent amino acid score[c]		73	73	73	73	73	73	73	73	73	73	91	97

[a] Data from FAO/WHO (1991). [b]Calculation of protein ratings of the ten amaranth accessions and a soybean cultivar (TGX 1448-2E) was carried out by comparison of the amino acid composition of hen's whole egg with that of the reference pattern established by FAO/WHO (1991) for a preschool child (2-5- year-old). [c] True protein digestibility values were taken from the USFDA (Federal Register, Appendix B (1993) and FAO (1973) scoring pattern.

Table 4. Amino acid scores of ten selected accessions of grain amaranth, one soybean cultivar (TGX 1448-2E) and their limiting amino acid.

EAA	EAA Requirement	2	3	9	10	17	18	21	23	27	28	30 (Soybean)
Histidine	19	2.36	1.61	2.22	2.36	2.34	1.86	1.77	2.38	2.47	2.26	2.78
Isoleucine	28	1.70	1.18	1.56	1.60	1.66	1.50	1.41	1.75	1.79	1.70	2.59
Leucine	66	1.14	0.81	1.05	1.08	1.09	0.99	0.91	1.16	1.17	1.12	1.85
Lysine	58	1.34	0.97	1.21	1.28	1.25	1.18	1.04	1.36	1.36	1.30	1.80
Methionine + cysteine	25	1.89	1.17	1.57	1.73	1.56	1.52	0.75	1.64	1.60	1.57	1.46
Phenylalanine + tyrosine	63	1.40	0.98	1.24	1.37	1.30	1.14	1.06	1.36	1.40	1.35	2.09
Threonine	34	1.42	1.03	1.33	1.35	1.32	1.24	1.13	1.41	1.5	1.41	1.97
Tryptophan	11	0.83	0.80	0.77	0.82	0.72	0.65	0.71	0.83	0.81	0.73	1.16
Valine	35	1.47	1.04	1.34	1.39	1.42	1.32	1.20	1.53	1.54	1.47	2.08
Limiting amino acid		Leu, Tryp	Leu, Tryp	Leu, Tryp	Leu, Tryp	Leu, Tryp	Leu, Tryp	Leu, Tryp	Leu, Tryp	Leu, Tryp	Leu, Tryp	Met, Tryp

Leu, Leucine; Try, tryptophan; Met, methionine.

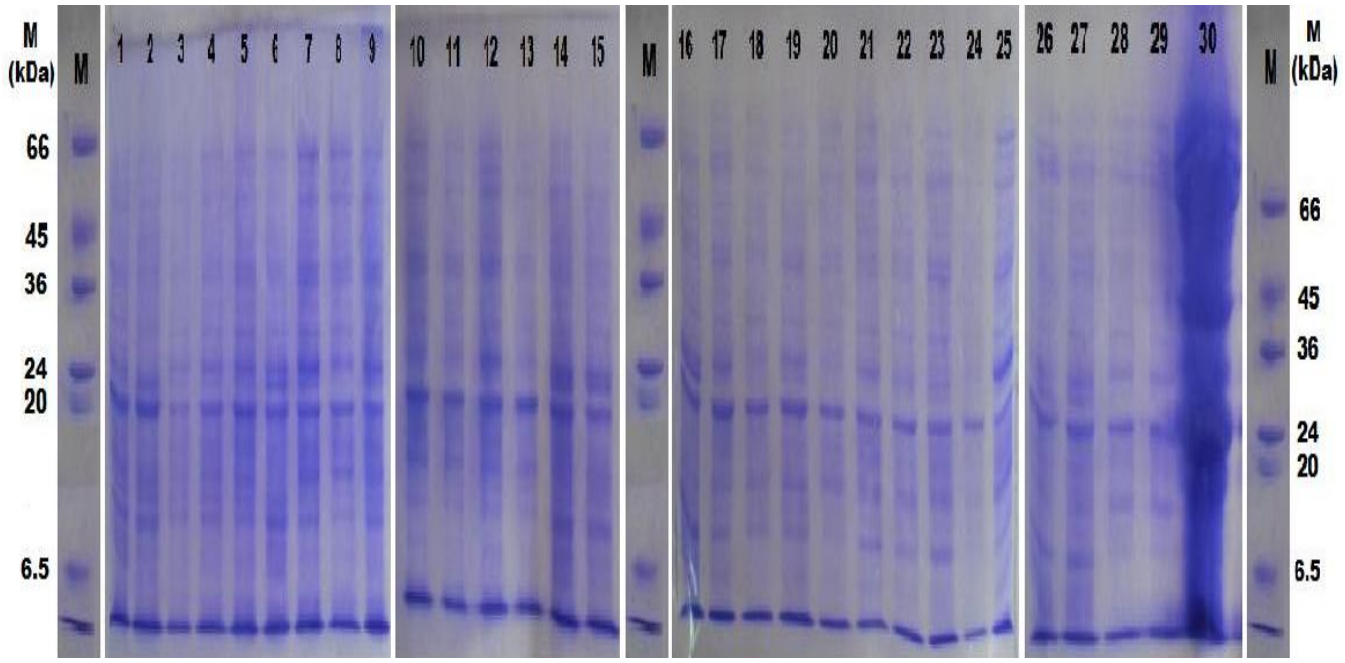

Figure 1. SDS-PAGE of albumin fractions of 29 accessions of *Amaranth* seeds. Lanes 1 - 4 *A. caudatus;* 5 - 12 *A. cruentus*; M-marker; 13 - 19 *A. hybrid*; 20 - 27 *A hypochondriacus*; 28 - 29, *A. hybridus*; 30, soybean (TGX 1448-2E).

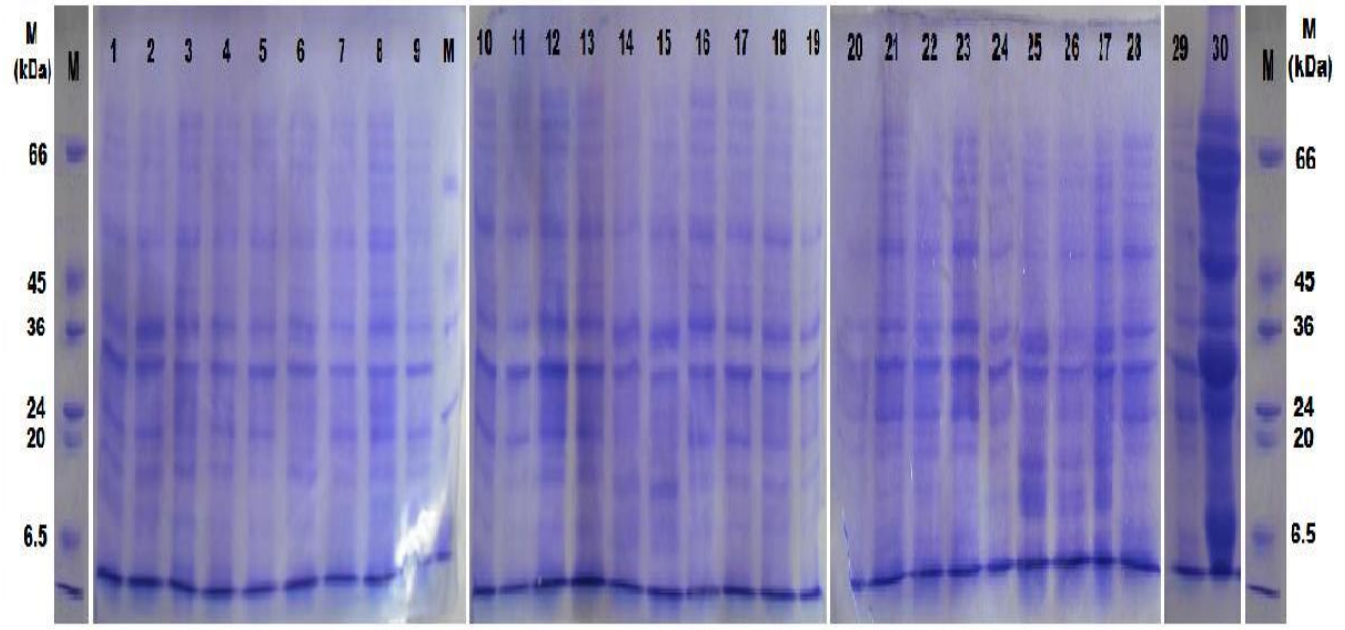

Figure 2. SDS-PAGE of globulin fractions of 29 accessions of *Amaranth* seeds. Lanes 1 - 4 *A. caudatus;* 5 - 12 *A. cruentus*; M-marker; 13 - 19 *A. hybrid*; 20 - 27 *A hypochondriacus*; 28 and 29, *A. hybridus*; 30 soybean (TGX 1448-2E).

Gorinstein et al. (1991b) who reported a major globulin band at 14 to 18 kDa of some amaranth species, but this is not in agreement with the result of Barba de la Rosa et al. (1992) who reported that all globulins showed a major band at 38 kDa. The amaranth glutelin fractions had three main non-separated subunits in the region of 4 to 14 kDa, 24 to 34 kDa and 65 to 66 kDa (Figure 3). These protein subunits were similar in all the 29 grain amaranth

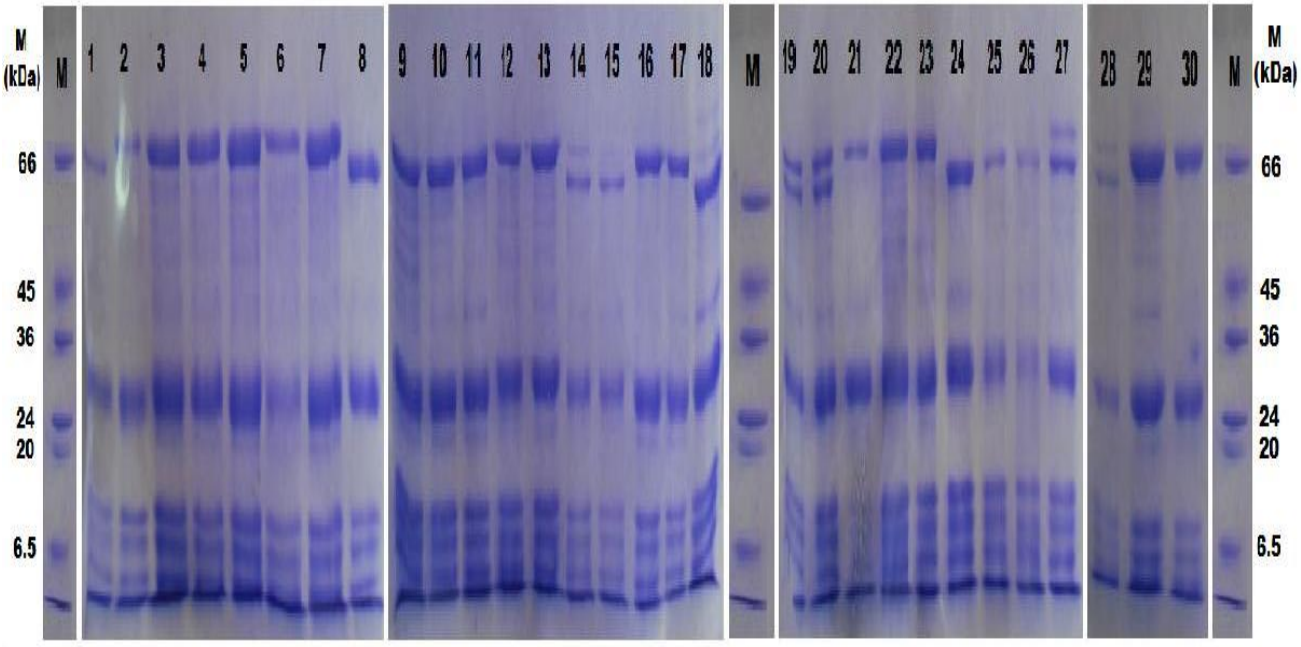

Figure 3. SDS-PAGE of glutelin fractions from 29 accessions of *Amaranth* seeds. Lanes 1 - 4 *A. caudatus;* 5 - 12 *A. cruentus*; 13 - 19 *A. hybrid*; M-marker; 20 - 27 *A. hypochondriacus*; 28 - 29, *A. hybridus*; 30 Soybean (TGX 1448-2E).

accessions. There were outstanding similarities between the electrophoretic patterns of all amaranth accessions evaluated and the soybean cultivar used for comparison. The abundant components in the glutelin fraction between 4 and 14 kDa, 30 kDa and 65 and 66 kDa of all 29 amaranth accessions evaluated in this study are similar to the results of Gorinstein et al. (2002) for *A. caudatus,* maize and rice. It will therefore be possible to use *Amaranthus* species in blends with other cereals for food nutrients.

Conclusion

Amaranth is a valuable food resource, containing a higher level of protein than cereals like maize, wheat, sorghum, rye and oat. It may therefore play an important role in human diets. Lysine and methionine which are limiting in most cereals are present in substantial high amount in amaranth. Most of the amaranth accessions had higher amounts of essential amino acids than the requirements of a 2-5-year old child and comparable amounts as whole egg protein. All accessions were high in non-essential amino acids such as glutamic acid, histidine, aspartic acid and glycine, limited only in tryptophan and leucine. Albumin, globulin and glutelin are the major fractions found in grain amaranth. Prolamin was not detected and so it is not a major fraction in amaranth. A close similarity between the glutelin fraction of amaranth and soybean was observed in the regions of 4 to 14 kDa, 30 kDa and 65 to 66 kDa. Amaranth could be a nutritive substitute for cereals and improve value in different diets in developing countries where protein malnutrition is still a major challenge.

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