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Biochemical and Nutritional Compositions of Two Accessions of *Amaranthus Cruentus* Seed Flour

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Abstract Amaranth is one of the few multi-purpose crops which can produce grains and tasty leafy vegetables of high nutritional quality as food for human consumption and animal feedstuff. Amaranth seeds have not recorded commercial success worldwide, due to the dominance of cereals and specific taste of amaranth leaves. However, previous research works have focused more on its cultivation. Therefore, the aim of this study was to determine the biochemical and nutritional composition of two accessions (PI538319 and PI538326) of *Amaranthus cruentus* seed flour and to evaluate the protein quality and the potential use of its flour to formulate functional foods and nutraceuticals, amino acid profiles of the samples were determined using the Technicon sequential multi-sample amino acid analyzer. The amino acid score was calculated with reference to amino acid composition of the whole egg, as well as FAO/WHO reference amino acid pattern. The two accessions of *Amaranthus cruentus (*PI538319 and PI538326) showed high protein values of 15.5 and 16.1%, while the values of essential amino acid recorded were 32.84 and 32.90 g/100g respectively. Lysine, which is known to be deficient in most cereals, was found to be relatively high with values of 3.50 and 3.71 g/100g respectively. The percentage ratio of the essential to non-essential amino acids was in the range of 42 – 45%. With reference to FAO/WHO (2007) standard, the chemical scores showed that most of the essential amino acids in the two accessions of *Amaranthus cruentus* were present in high amounts when compared with other grain sources. Thus, with the nutritional composition and amino acid profiles, it shows that this seed flour can fulfill the protein requirements of an adult human being.

Keywords: *nutritional composition, amino acid profile, protein supplement*

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1. Introduction

The genus *Amaranthus* includes about 60 species, most of which are cosmopolitan weeds associated with difficulties in cultivation practices after soil disturbance and seed exposure to light [1,2]. The three principal series of the genus *Amaranthus*, originating in South America and considered for grain production and domesticated abroad are *A. hypochondriacus* L.(Mexico), *A. cruentus* L.(Guatemala), and *A, caudatus* L. (Peru and other Andean countries) [1,3,4,5]. The structure of grain amaranth, a pseudocereal, differs significantly from other pseudocereals and cereals. Amaranth seeds are small (0.9 – 1.7 mm of diameter) with 1000 seed weights from 0.6-1 g. They are lenticular in shape and the seed colour varies from gold, white, pink and brown to black [2,4].

In amaranth, the embryo is campylotropous and surrounds the starch-rich perisperm like a ring and together with the seed coat represents the bran fraction, which is relatively rich in fat and protein [6]. The percentage of bran fraction is higher in amaranth when compared with common cereals, which explains the higher level of protein and fat present in the seeds. In amaranth, the seed coat is smooth and thin, thus it is not necessary to remove it [1,4]. The nutritional value of amaranth is mainly connected to their proteins that constitute an important parameter of bio-macromolecules involved in physiological functions. According to literature reports, the protein content is 14-16.5% for amaranth [6,7,8]. Compared with 12.9 – 16.5% for quinoa and common cereal grains, the protein content (average of 14.60% and 13.80% respectively) is significantly higher than that of maize (10.20%), and comparable to that of wheat (14.30%) [9].

Natural vegetable proteins are useful owing to their safety, high biocompatibility, nutritional value and low cost. Finding new vegetable proteins that are rich in essential amino acids is important for food and pharmaceutical industries [10]. Most cereals are deficient in some amino acids; for instance corn is deficient in lysine and tryptophan, rice in lysine [11] and wheat in lysine [12]. Amaranth (*Amaranthus*) belongs to a nutritious class of pseudocereals 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 [13]. Contrary to common grains such as wheat, the proteins are composed mainly of globulins and albumins, with very little or no storage prolamin proteins, which are the main storage proteins in cereals and major components of the toxic proteins, implicated in celiac disease development [2,5,14]. Because of the very little or no prolamin content, amaranth seeds are considered to be gluten-free grains. Amaranth proteins consist of about 40% albumins, 20% globulins, 25 – 30% glutelins, and 2 -3% prolamins [15].

Protein content and amino acid composition depend on genotype and growing conditions [16]. Most investigations on amaranth species have focused on storage protein fractions. However, assessment of the nutritional quality of *Amaranthus cruentus* based on total protein content, and amino acid composition with reference to amino acid requirement for human is scarce, according to [17]. Thus, this study was carried out to determine the biochemical and nutritional compositions of two accessions of *Amaranthus cruentus* PI538319 and PI538326 seed flour. Findings from this study would assist our food product developers and nutritionist for better utilization of seeds.

2. Materials and Methods

2.1. Samples

Two accessions of *Amaranthus cruentus* with accession code PI538319 and PI538326 which were used in this study was sourced from Nigeria Horticultural Research Institute (NIHORT) germplasm, Ibadan, Nigeria. These two accessions were planted in the experimental field of the Federal University of Technology, Akure in 2015. It was planted in three replicates in a randomized complete block design. The seeds were harvested at maturity [10-12 weeks].

2.2. Sample Preparation

The whole seeds of each harvested accession were pooled together, winnowed and ground in a mill. The resulting flour was packaged and stored at 4°C prior to further analysis.

2.3. Methodology

The AOAC [18] standard method was used to determine the proximate composition. T he carbohydrate by difference was obtained as described by [19]. Energy value was obtained by the method of [20]. Each analysis was carried out in triplicate.

2.4. Amino Acid Analysis and Nutritional Quality Evaluation

The amino acid profile of the *A. cruentus* accessions was determined using the Technicon sequential multi-sample amino acid analyzer. Prior to analyses, samples were hydrolysed for 22 hrs at 105 ± 5°C with 6 M HCl by the method of [21] as reported by *[22]*. Nutritional quality of the seed flour was estimated by determination of ratio of essential (E) to non-essential (NE) amino acid, amino acid score, protein efficieny ratio, hydrophilic and hydrophobic content. The amino acid score was calculated with reference to amino acid composition of the whole egg [23], as well as FAO/WHO [17] reference amino acid pattern.

$$\text{Amino acid score} = \frac{\text{Testamino acid}}{\text{Reference amino acid}} X100 \quad (1)$$

Nutritional qualities were determined on the basis of the amino acid profiles. The Essential Amino Acid Index (EAAI) was calculated using the method of [24] according to the equation below:

$$\text{EAAI} = n\sqrt{\frac{100a \times 100b \ldots 100j}{av \times bv \ \ldots jv}} \quad (2)$$

Where:

n = number of essential amino acids, a, b, …j = represent the concentration of essential amino acids (lysine, tryptophan, isoleucine, valine, arginine, threonine, leucine, phenylalanine, histidine and the sum of methionine and cysteine) in test sample and av, bv … jv = content of the same amino acids in standard protein (%) (Egg or casein) respectively. Biological value was calculated according to [25] cited by [26] using the following equation:

$$BV = 1.09 \times \text{Essential amino acid index } (\text{EAAI}) - 11.7. \quad (3)$$

The predicted protein efficiency ratios (PER) values of the different accessions was calculated from their amino acid composition according to the regression equations developed by [27] cited by *[26]* as given below:

$$PER = -0.486 + 0.454(LEU) - 0.105(TYR). \quad (4)$$

2.5. Statistical Analysis

Analysis of variance and Duncan's multiple range tests were done according to the Statistical Analysis System package [28].

3. Results and Discussion

3.1. Proximate Composition

The proximate compositions of flours of the two accessions of *Amaranthus cruentus* (PI538319 and PI538326) seed are shown in Table 1 with significant differences ($p>0.05$) between both flours. The moisture ranged between 7.2 and 8.3% which is higher than the values reported for *Phaseolus lunatus* 3.17 – 4.96% [29], and 6.45% reported for Bambara groundnut [30] but lower than 10.39% reported for cowpea and 8.30% for mungbean [31]. The major quality factor in the preservation of some food products and produce is moisture as its affects food stability [32], which could be an indication of good keeping quality and shelf life of the seeds constituents. These accessions showed crude protein contents that ranged between 15.2 – 16.1%, which are higher than what is obtainable in most cereals crops. The values are higher than those recorded for corn (9%), rye (13%), buckwheat (12%), rice (7%), human milk (3.5%) [33], and quinoa (15%) [34]. This gives an indication that amaranth grain is a good source of dietary protein supplement to meet recommended daily requirement for humans [35]. The high protein contents of the amaranth seeds flour as a pseudocereal, underline its potential as protein supplements to improve protein quality in composite flour. Amaranth grain is considered to have a unique composition of proteins, carbohydrates and lipids; thus it can provide a balanced nutrient uptake with lower amounts of consumption when compared to cereals. Previous research works have reported amaranth grains to contain about % protein and 60% starch with good amino acid profile that makes it a balanced protein source [36]. The values are in agreement with those reported by previous researchers for *A. cruentus* species. "Reference [37,38,39,40]" reported values ranges of crude protein 12 – 19%, fat 5 – 8%, crude fiber 3 – 5%, and ash 2 – 4% on dry matter basis. The calculated metabolizable energy values showed that all the grains used in this study are concentrated sources of energy.

Table 1. Proximate composition of two accessions of *Amaranthus cruentus* (PI538319 & PI538326) flours

Sample	PI 538319	PI 538326
Crude protein (%)	15.5[b]	16.1[a]
Moisture content (%)	8.3[a]	7.2[b]
Total ash (%)	3.1[a]	2.5[b]
Crude fat (%)	8.0[a]	7.8[b]
Crude fiber (%)	3.9[b]	4.1[a]
*Carbohydrate (%)	61.2[a]	62.3[a]
Energy value (MJ kg^{-1})	16.0903	15.8554

*= Calculated by difference. Values followed by different letters in a row are significantly different (p>0.05).

3.2. Amino Acid Composition

The amino acid profile of the flour of two accessions of *A. cruentus* PI538319 and PI538326 seeds (Table 2) showed little variation in the content of total essential amino acid (EAA) and non-essential amino acid (NEAA). The percentage ratio of EAA to total amino acid (TAA) was in the range 42 – 44%, which is above 36% considered adequate for an ideal protein [17]. Glutamic acid was the most abundant amino in PI538319 and PI538326, with 9.31 g/100 g and 10.52 g/100 g levels, respectively. The second most abundant amino acid is aspartic acid (8.25 g/100 g and 8.31 g/100 g). The most concentrated essential amino acids in the amaranth flour is Leucine with values of 4.96 and 5.19 g/100 g respectively while the least concentrated is Methionine with values of 0.75 and 0.80 g/100 g. Glutamic was found to be the major non-essential amino acid in this study. The nutritive value of a protein depends primarily on the capacity to satisfy the needs for nitrogen and essential amino acids [41]. The PI538319 and PI538326 flour samples contain high concentrations of lysine in the range of 3.55 – 3.71 g/100 g total protein. Lysine is a nutritionally critical amino acid and an essential amino acid that is lacking in all of the world's main cereal crops [40]. The total essential amino acids (TEAA) values (with histidine) ranged between 32.84% in PI538319 to 32.90% in PI538326 (Table 3). These values are comparable with those reported for some selected oil seeds, which ranged between 33.3 and 53.6% [42], but higher than the values reported by [43] for raw popcorn flour 26.55%. The results suggest that these studied pseudocereals can be used as food supplements; hence its commercial cultivation should encourage in order to ensure a secured food environment.

Table 2. Amino acid composition of two accessions of *Amaranthus cruentus* (PI538319 & PI538326) (g/100g)

Amino acid	PI538319	PI538326
Threonine*	3.49	3.19
Tyrosine*	2.23	2.23
Methionine*	0.75	0.80
Valine*	3.27	3.33
Phenylalanine*	3.01	3.19
Isoleucine*	2.55	2.09
Leucine*	4.96	5.19
Lysine*	3.55	3.71
Cysteine*	0.78	0.84
Histidine*	2.04	2.20
Tryptophan*	1.05	0.97
Arginine*	5.16	5.16
Aspartic acid	8.25	8.31
Glutamic acid	9.31	10.52
Serine	3.10	3.54
Proline	2.64	2.84
Glycine	3.37	3.46
Alanine	3.33	3.49

*Essential Amino Acids.

The ratio of total essential to non-essential amino acids was 1.10 and 1.02 for the PI538319 and PI538326, respectively. Essential aliphatic amino acids (EAAA), isoleucine, leucine and valine, which constitute the hydrophobic regions of proteins, were not abundantly present in the flour. Table 3 also show the percent of total acidic amino acids (TAAA), which was found to be greater than the percent of total basic amino acids (TBAA) in both samples indicating that the protein is mainly acidic in nature. The protein efficiency ratio of the samples was 1.53 g/100 g and 1.64 g/100 g for PI538319 and PI538326, respectively. The essential amino acid indices (EAAIs) of the flour were 29.07% and 26.33% for PI538319 and PI538326, respectively while the biological values (BV) were 19.09% and 16.20%. According to Oser [25], a protein material is said to be of good nutritional quality when its BV is high (70 – 100%) and also when the EAAI is above 90% and to be useful as food when the values is around 80% and to be inadequate for food material when below 70%. From the present study it was observed that the BV and EAAI values were generally low; and these could be attributed to the fact that amaranth, a pseudocereal, is low in protein content compared with the legumes. However, previous investigations have shown that amaranth is notably rich in lysine and tryptophan, which are nutritionally important amino acids [40]. Therefore this attribute of amaranth protein can be used as a blending food source to increase the biological value of processed foods [44]. This may impart health benefits or desirable physiological effects and can be used to develop new food products with beneficial components. A number of researchers have uncovered an important conclusion: the protein value (protein efficiency ratio) of the raw amaranth grain does not reflect the amino acid pattern of the protein.

Table 3. Nutritional quality and classification of amino acids (g/100g) found in two accessions of *Amaranthus cruentus* (PI538319 and PI538326)

Amino acid description	*PI538319(g/100g)*	*PI538326(g/100g)*
Total proteins	15.5	16.1
Total amino acids (TAA)	62.84	65.06
Total essential amino acids (TEAA) with histidine	32.84	32.90
Total essential amino acids (TEAA) without histidine	30.80	30.70
% TEAA with histidine	52.26	50.57
% TEAA without histidine	49.01	47.19
Total non-essential amino acids (TNEAA)	30.00	32.16
% TNEAA	47.74	49.43
TEAA/TNEAA	1.10	1.02
Essential aromatic amino acids (EArAA)	5.24	5.42
Total neutral amino acids (TNAA)	24.93	26.40
% TNAA	39.67	40.58
Total acidic amino acids (TAAA)	17.56	18.83
% TAAA	27.94	28.94
Total basic amino acids (TBAA)	10.75	11.07
% TBAA	17.11	17.02
Total sulphur containing amino acids (TSAA)	1.53	1.64
% Cysteine in TSAA	50.98	51.22
Essential aliphatic amino acids (EAAA)	10.78	10.61
Protein efficiency ratio (PER)	1.53	1.64
Biological value (BV)	19.99	16.20
Essential amino acids index (EAAI)	29.07	26.33

Table 4. Amino Acid Scores of *Amaranthus cruentus* accessions PI538319 & PI 538326

Amino acids	Reference FAO/WHO standard (2007, mg/g)	EAAC PI538319 (mg/g)	AAS PI538319 (mg/g)	EAAC PI538326 (mg/g)	AAS PI538326 (mg/g)
Lysine	45	35.5	78.9	37.1	82.4
Histidine	15	20.4	136	22	146.7
Threonine	23	34.9	151.7	30.9	134.4
Valine	39	32.7	83.9	33.3	85.4
Isoleucine	30	25.3	84.3	20.9	69.7
Leucine	59	49.6	84.1	51.9	88
Methionine + Cystine	22	15.3	69.6	16.4	74.6
Phenylalanine + Tyrosine	30	52.4	137.9	54.2	146.6
Chemical score			865.8		869.1
1st limiting amino acid			Methionine + Cysteine		Isoleucine
2nd limiting amino acid					Methionine + cysteine
	Whole egg pattern				
Lysine	70	35.5	50.7	37.1	53
Histidine	22	20.4	92.7	22	100
Threonine	47	34.9	74.3	30.9	65.8
Valine	66	32.7	49.6	33.3	50.5
Isoleucine	54	25.3	47.2	16.4	28.8
Leucine	66	49.6	75.2	20.9	38.7
Methionine + Cysteine	57	15.3	26.8	51.9	78.6
Phenylalanine + Tyrosine	93	52.4	56.3	54.2	58.3
Chemical score			472.8		473.7

EAAC = Essential Amino Acids composition, AAS = Amino Acids Scores.

Furthermore, the amino acid chemical score of *A. cruentus* were compared with reference to [17] amino acid reference pattern for adult diet and to amino acid composition of the whole egg protein, which is considered to be a complete and balanced food [22]. With reference to FAO/WHO standard, the chemical score showed that most of the essential amino acids were in excess (Table 4). But with reference to whole egg protein only histidine was in excess in both samples respectively. In addition, the limiting amino acid in PI538319 was found to be the sulphur-containing amino acids while the first and second limiting amino acids in PI538326 were found to be isoleucine and sulphur-containing amino acids. The remaining essential amino acids are available in excess. *Amaranthus cruentus* accession code PI538319 and PI538326 are found to be deficient in sulphur containing amino acids and excess in aromatic amino acids. This study is in contrast to the research done by [45] and [44], which was reported by [40] that studies have shown leucine to be the first limiting amino acid in amaranth but which was found to be in excess in this study. The reason may be due to different planting seasons, variation in the soil nutrients, environmental factors and geographical locations, among others.

Furthermore, the essential amino acids of both flour samples were compared with [17] (Table 5); it was observed that the amino acid compositions of PI538319 and PI538326 were higher than the reference standard except for leucine, isoleucine, methionine, and valine. The relatively high content of essential amino acids in amaranth grain is favourable for its use as a substitution for meat-and-bone food/feed [46] complementary weaning food [40].

Table 5. Comparison of FAO/WHO amino acids recommended and the amaranths PI538319 and PI538326 amino acids composition

EAA	FAO/WHO	PI538319	PI538326
Arginine	NR	5.16	5.16
Histidine	1.5	2.04	2.20
Isoleucine	3	2.55	2.09
Leucine	5.9	4.96	5.19
Lysine	4.5	3.55	3.71
Methionine	1.6	0.75	0.80
Phenylalanine	NR	3.01	3.19
Threonine	2.3	3.49	3.19
Tryptophan	0.6	1.05	0.97
Valine	3.9	3.27	3.33
TSAA (Met + Cys)	2.2	1.53	1.64
TArAA (Phe +Tyr)	3.0	5.24	5.42
TEAAs	27.0	32.84	32.90

Source: FAO/WHO 2007. Total sulfur amino acids (TSAAs), Total aromatic amino acids (TAAAs), Total essential amino acids (TEAAs), EAA= Essential Amino Acids, NR = Not Reported.

4. Conclusion

The research work revealed that the essential amino acid composition compared favourably with the reference FAO/WHO standards making the amaranth flour a more suitable non-wheat material for composite flours used for fortified food and complementary weaning food production respectively. Furthermore, based on its protein quality it can be used in food product development for celiac patient. In addition, the lysine content which is limiting in many cereals is available in abundant in this two accessions making it serves as an excellent source of protein with potential to complement poor quality protein cereals e.g. rice, sorghum, millet, wheat among others. Finally, this study is meant to give amaranth grains a future in Nigeria and expose its potential utilizations.

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