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Full Length Research Paper

Amino acid profile of *Amaranthus caudatus*

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Fresh leaves of *Amaranthus caudatus* were collected and pretreated for amino acids profile with the sole aim of studying the essentiality of this leaves in food consumption in Adamawa State, Nigeria. The results obtained from the analysis shows that seventeen amino acids were present in varying concentration in the vegetable. Glutamic acid, leucine, aspartic acid and valine have considerable high concentration; 11.77 g/100g protein, 9.39 g/100g protein, 7.92 g/100g protein and 5.00 g/100g protein respectively. Cysteine and methionine are the limiting essential amino acids in the vegetable with 0.93 g/100g protein and 1.09 g/100g protein respectively. Most of the essential amino acids compared favorably with FAO/WHO standards.

Key words: *Amaranthus caudatus,* vegetable, protein, leaves, valine.

INTRODUCTION

Vegetable is defined as the leafy outgrowth of plants used as food and others part used in making soup or served as an integral part of the main source of meal. (Alfred and Patrick, 1987). The status of food as a good source of protein is usually judged from its amino acid contents in right proportions capable of promoting growth when it is the sole source of protein in the diet (Akpabio *et al.,* 2008). This was achieved through research strategies employed to fight against malnutrition, under-nourishment and nutritional supplement desired earnestly by man which has been the basic goal for any national development. Thereby analyzing the nutritional quality and chemical composition of different varieties of green leafy vegetable, most particularly the Amaranth family (Aguire and Borneo, 2008).

Vegetables are the fresh and edible portions of herbaceous plants. They are important class of food substances and highly beneficial for the maintenance of health and prevention of diseases. They contain valuable food ingredients which can be successfully utilized to build up and repair the body. They are valued mainly for their high carbohydrates, vitamins and mineral contents. There are different kinds of vegetables. They may be edible roots, stems, leaves, fruits and seeds. Each group contributes to diet in its own way. Vegetables as a whole are considered as natural sources of nutrients gifted by God to human beings, for example, carrot is a good source of vitamin A needed for normal vision; likewise spinach and tomato contain enough amount of vitamin C to prevent an cure scurvy. Potato is rich in starch and provides high amount of carbohydrates. More recent epidemiological studies have supported the association between better health and long term consumption of diets rich in vegetables (Rumeza et al., 2006; Hung et al., 2004; Jansen et al., 2004).

The viable economic and nutritional advantage of the amaranth species as a leaf vegetable is primary based on its agronomic superiority over many plant leave protein source for instance harvesting is done within 15-30 days after transplanting and then 2-3 weeks for a period of 1 to 2 months (Leung et al., 1992). Another potential nutritional advantage of the amaranth grains compared to convectional cereals is a relative high content of proteins and more balance composition of essential amino acids, this highly favor the plant leaves as a veritable source of protein (Carlson, 1979; Bressani et al.,1987). In developing nations, numerous types of edible wild plants are exploited as sources of food and provide adequate level of nutrients for the inhabitants. Some studies in some societies in Africa indicate that these plants resources play a significant role in nutrition; food security and income generation (Asibey-Berko and Tayie, 1999).

Research has shown that eaten enough diet of

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vegetable within the caloric needs help to maintain sound health and reduce blood pressure, heart disease, diabetes, cancer and building of body defense immunity (Akpabio *et al.,* 2008). The nutritional values found in green vegetable include water, mineral, phytochemicals and protein.

Therefore, the aim of present study was to identifying the individual amino acids present in the green leaves of *Amaranthus caudatus.*

MATERIALS AND METHODS

Sample collection and preparation

In this study, *Amaranthus caudatus* plants were collected from Gerei, Adamawa State, Nigeria. The leaves of the plants were air dried at room temperature and grinded into powder using mortar and pestle.

Amino acid analysis

The amino acid profile in the known sample was determined using methods describe by (Sparkman et al., 1958).The sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotator evaporator and loaded into the Technicon Sequential Multi-Sample Amino Acid Analyzer (TSM) (Technicon Industries System, Tarrytown, New York).

Defatting Sample

The dried ground sample was weighed into extraction thimble and the fat was extracted with chloroform/methanol (2:1 mixture) using Soxhlet extraction apparatus as described by AOAC (2006) the extraction lasted for 15 hrs.

Crude protein determination

A small amount (200mg) of ground sample was weighed; wrapped in whatman filter paper (No 1) and put into the kjeldahl digestion flask. Concentrated sulphuric acid (10ml) was added .catalyst mixture (0.5g) containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$), and selenium oxide (SeO_2) in the ratio of 10:5:1was added into the flask to facilitate digestion. Four pieces of anti- bumping granules were added.

The flask was then put in kjeldahl digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100ml in standard volumetric flask. Aliquot (10ml) of the diluted solution with 10ml of 45% sodium hydroxide was put into the Markham distillation apparatus and distilled into10ml of 2% boric acid containing 4drops of bromocresol green/methyl red indicator until about 70ml of distillate was collected.

The distillate was then titrated with standardize 0.1M hydrochloric acid to grey colored end point, the percentage of nitrogen in the original sample was calculated using the formula:

$$Percentage\ Nitrogen = \frac{(a-b) \times 0.01 \times 14 \times V \times 100}{W \times C}$$

Where:

a = Titre value of the digested sample
b = Titre value of blank sample
v = volume after dilution (100ml)
w = weight of dried sample (mg)
c = Aliquot of the sample used (10ml)
14 = Nitrogen constant in mg

Hydrolysis of the sample

A known weight of the defatted sample was weighed into glass ampoule. Seven ml of 6M HCl was added and oxygen was expelled by passing nitrogen into the ampoule (this is to avoid possible oxidation of some amino acids during hydrolysis e.g. methionine and cysteine), the glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105^{o}C ± 5^{o}C for 22hours. The ampoule was allowed to cool before broken open at the tip and the content was filter to remove the humins. It should be noted that tryptophan is destroyed by 6M HCl during hydrolysis.

The filtrate was then evaporated to dryness at 40^{o}C under vacuum in a rotator evaporator. The residue was dissolved with 5ml to acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer.

Loading of the sample into TSM analyzer

The amount loaded was between 5 to 10 microlitres. This was dispended into the cartridge of the analyzer. The TSM analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. The period of the analysis lasted for 76 minutes.

Method of Calculating Amino Acid Values from the Chromatogram Peaks

The net height of each peak produced by the chart recorder of TSM (each representing an amino acid) was measured. The half- height of the peak on the chart was found and the width of the peak on the half height was accurately measured and recorded. Approximately area of each peak was then obtained by multiplying the height with the width at half-height.

The norleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula.

$$NE = \frac{\text{Area of Norleucine peak}}{\text{Area of each amino acid}}$$

A constant S was calculated for each amino acid in the standard mixture: where $S_{std} = NE_{std} \times$ Molecular weight $\times \mu MAA_{std}$

Finally, the amount of each amino acid present in the sample was calculated in g/16gN or g/100g protein using the following formula:

Table 1. Standard run for the amino acid analysis

1	2	3	4	5	6	7	
AMINO ACID	**NET HEIGHT (mm)**	**NH/2 (mm)**	**Width at NH/2 (mm)**	**NH x W**	**NEstd**	**Molecular weight**	**S**
Lysine	87	43.5	4.5	391.5		182.65	12.92
Histidine	77	38.5	5.0	385	2.887	209.63	15.08
Ammonia						53.49	
Arginine	26.5	13.25	20			174.20	9.10
Aspartic acid	86	43	5			133.11	8.57
Threonine	86.5	43.25	5			119.12	7.63
Serine	98	49	4			105.00	7.42
Glatamic acid	62	31	5.5			147.13	11.95
Praline	23	11.5	9.5			115.13	14.60
Glycine	79	39.5	4.5			75.09	5.85
Alanine	59	29.5	4.5			89.09	9.29
Cystine	39	19.5	19			240.30	9.10
Valine	99.5	49.7	3.5			117.15	9.32
Methionine	103	51.5	4			149.21	10.03
Isoluecine	93	46.5	4.5			131.18	8.68
Leucine	107	53.5	4.5			131.18	7.55
Norleucine	138.5	69.23	8	1108		131.18	
Tyrosine	36	18	9			181.18	15.50
Phenylalanine	37.5	18.75	12			165.19	10.17

E.G NE $_{std}$ (Lysine) = $\frac{138.5 \times 8}{87 \times 4.5} = 2.830140485$

Note: 1: µmoles AA = 0.025

2: $NE_{sid} = \frac{NH\ X\ W\ (nLED}{NH\ X\ W(AA)}$

3: S = NE_{std} x MOL, Weight xμMAA_{std}

Concentration (g/100g protein) = NH × w at NH/2× S_{std} × C

Where C

$$= \frac{\text{Dilution} \times 16}{\text{Sample Wt (g)} \times N\% \times 10x \text{ vol. loaded}} \div NH \times W\ (nle\mu)$$

Where: NH=Net height
W=Width at half height
nlµe= Norleucine

RESULTS AND DISCUSSION

Table 1 shows the standard run. The highest peak is norcleucine with height of 138.5 mm the short peak is proline with height of 23 mm, the widest peak is arginine with width of 20mm and the constant with the highest value of amino acid is tyrosine with a value of 15.50 and the lowest is glycine with the value of 5.85.

Table 2 shows the result of sample run for the *Amaranthus caudatus,* the highest peak is leucine with height of 171 mm, the shortage peak is cysteine and tyrosine with height of 7 mm of 18 mm, the amino acid with highest concentration of g/100g protein is glutamic acid and the amino acid with lowest concentration is cysteine with 0.93/100g protein.

MONDEL DNA 0209% N(Fat free) =3.83

Wt. of sample hydrolysis = 5222g volume loaded: basis 10µL

Dilution = x 5 Acid/neutral - 5µL

Concentration (g/100g protein) = NH x NH/2 x S_{std} x C

C_{basis} = 0.001038947

Where S_{std} =NE_{std} x Mol. Weight x μAA_{std} C $_{Acid}$

C/neutral = 0.002077894

$$C = \frac{\text{Dilution x 16}}{sample\ Wt(g) x\ N\% \ x\ 10\ x\ vol.loaded} \div NH \text{ x } W \text{ (nleu)}$$

Table 2. Result of amino acid analysis in *Amaranthus caudatus*

1	2	3	4	5	6=(2x4x5xC)
Amino acid	**Net height**	**NH/2 (mm)**	**Width at NH/2 (mm)**	**S_{std}**	**Concentration: g/100g protein**
Lysine	85	42.5	4	12.92	4.56
Histidine	39	19.5	4	15.08	2.44
Ammonia					
Arginine	25	12.5	18	9.10	4.25
Aspartic acid	127	63.5	3.5	8.57	7.92
Threonine	56	28	3.5	7.63	3.11
Serine	45	22.5	3.5	7.42	2.43
Glutamic acid	79	39.5	6	11.95	11.77
Proline	11	5.5	7	14.60	2.34
Glycine	63	31.5	4	5.85	3.06
Alanine	53	26.5	4	9.29	4.09
Cysteine	7	3.5	7	9.10	0.93
Valine	86	43	3	9.32	5.00
Methionine	21	10.5	2.5	10.03	1.09
Isoleucine	58	29	3.5	8.68	3.66
Leucine	171	85.5	3.5	7.55	9.39
Norleucine	70	35	5.5		
Tyrosine	7	3.5	10	15.50	2.55
Phenylalanine	21	10.5	8	10.17	3.55

Table 3. Comparison of essential amino acids in *Amaranthus caudatus* with WHO/FAO standard in g/100g protein

Amino acid	WHO/FAO standard	*Amaranthus caudatus*
Lysine	4.20	4.56
Threonine	2.80	3.11
Cysteine	2.00	0.93
Valine	4.20	5.00
Methionine	2.20	1.09
Isoleucine	4.20	3.66
Leucine	4.20	9.39
Tyrosine	2.80	2.25
Phenylalanine	2.80	3.55

Calculation for each amino acid present in the sample in g/100g protein.

Concentration (g/100g protein) = Net height x width at $\frac{NH}{2}$ x S_{std} x C

Where C = C_{basis} (0.001038946) or C_{Acid}C/Neutral (0.002077894)

Therefore concentration: g/100g protein

Lysine = 85 x 4 12.92 x 0.001038947 = 4.563886382 ≈ 4.56

Concentration g/100g $protein_{(Histidine)}$ = 39 x 4 x 15.08 x 0.001038947 = 2.444102039 ≈ 2.44

Seventeen amino acid were found in varying proportions in the vegetables. Most of the essential amino acids were present. The proportions of the essential amino acids in the vegetables were compared with the WHO/FAO protein standard. From Table 3, the essential amino acid in both vegetables was compared favorably with the WHO protein standard.

The analyses of the amino acid content of the leave vegetable indicated on Table 2 as shown above, eighteen amino acid were found in varying proportions in the vegetables, all the essential amino acid were confirmed present but the proportion of each of the essential amino acid in the vegetable were compared with WHO/FAO protein standard. Lysine, threonine, valine, leucine and phenylalanine are of high values with slight difference in value compared to that of WHO/FAO standard.

This shows that the vegetable is highly rich in protein as a source of amino acid while cysteine, methionine, isoleucine and tyrosine which consist of the value less than the required standard also serve as good, quality source of protein to human by consuming such vegetable

with food that are rich in protein as a source of nutrient supplement.

Conclusion

The analyzed work has revealed that vegetable *Amaranthus Caudatus*, commonly consumed in Adamawa state and Nigeria at large is rich in protein which serve as source of amino acid with the exception of tryptophan which is absent, too low for detection in the sample or it is destroyed by chemical during hydrolysis. This re-enforce the growing awareness that wild and semi-wild vegetable can contribute useful amount of essential nutrient to human diet.

Therefore when compared with WHO/FAO reference standard the nutritional value of the vegetable were confirm good, and essentially needed to lower cholesterol level in the body, sugar concentration, repair of muscle and generally improve the health and well-being of the body (Barminas et al., 1998; Kubmarawa et al., 2009; BNF, 1990). Although the vegetables are rich in nutrients lack of tryptophan from them means they should be consumed together with source of tryptophan and other class of food nutrient, as source of balance diet needed for the body at right proportion. The level of arginine and histidine in the vegetable warrants them to be recommended for children food since children need arginine and histidine in their foods.

RECOMMENDATION

Further work is recommended to investigate the vitamin content of this vegetable and other trace minerals which are not covered in this present work. Anti nutritional factors and toxicant which may turn the nutrients determined in this work inaccessible and invalid to the intended consumers should be accessed for the fear of side effects.

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