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Amino acid Composition, Amino Acid Scores and Predicted Protein Efficiency Ratio of Raw and Cooked African Yam Bean (*Sphenostylis sternocarpa*)

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The amino acid composition of raw and heat processing was analyzed by amino acid analyzer. The patterns of 7 essential amino acids were reported in g per 100 g bean protein and the amino acid score and predicted protein efficiency ratio were calculated and reported. The protein quality of the four products were predicted by calculating protein efficiency ratio. Results indicated that processing had various effects which were in this order: roasting > microwave cooking > conventional cooking. Total amino acid values were 78.25, 67.57, 72.25 and 80.0 g/100 g protein for flour from raw, conventionally cooked, microwave cooked and roasted samples respectively. Essential amino acids namely valine, methionine and phenylalanine in both raw and processed samples were not sufficient to meet human nutritional needs based on FAO/WHO (1991) reference pattern for amino acids. The predicted protein efficiency ratio (P-PER) was 2.26 for flour from the raw sample while P-PER of flour from conventionally cooked, microwave cooked and roasted samples were 2.05, 2.19 and 2.31 respectively. The present study indicated that total and particularly essential amino acids contents and amino acid composition of the flour samples changed by the processing methods. Among them, roasting enhanced the contents of amino acids in comparison to conventional cooking and microwave cooking.

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1. INTRODUCTION

Leguminous seeds are good sources of plant proteins [1]. They are nutritious foods and a substitute for an animal protein which arises from the knowledge of the functional properties of the seed flour and other products [2]. With high malnutrition in Africa due to insufficient animal protein, there is an intensive search for alternative sources of protein from minor proteins [3]. Their nutritional and functional properties dramatically affect the overall quality and its technological performance [4].

Foods are processed by various means to get them to a state the body can absorb nutrients maximally. For the legumes, they contain anti-nutritional factors like protease inhibitors, phytates, oxalates, saponins which inhibit or limit maximum absorption of amino acids from them. However, there is a remarkable improvement in the nutritive value and quality of legume seeds which have been achieved through de-hulling, heat treatment, germination, fermentation, soaking and partial hydrolysis by proteolytic enzymes [5]. Heat treatments employed in food processing include roasting, grilling, boiling/cooking, microwave cooking, ohmic heating, baking, toasting, frying, etc. These processing methods may have the potentials of reducing antinutritional factors which interfere with protein digestibility and amino acid absorption. The reason stems from the fact that protein quality is defined by its amino acid composition and this influences nutrients derived from them [6].

Sphenostylis sternocarpa (African yam bean) is a legume found in the tropics. It is called 'odudu', 'Ukpodudu', 'Okpodua' [1], 'Azuma' by some Igbo clans 'Bebe' by the Yorubas and in Northern states of Nigeria 'Kashin kaji' [7]. It is a leguminous crop of the family Leguminosae and sub-family Papilionaceae [1,8]. It is a herbaceous climbing vine which produces ellipsoid, round or truncated seeds which vary in size and colour ranging from creamy-white or brownish yellow to dark brown [1]. This work was aimed at studying the changes effected by various heating methods on amino acid composition of *S. sternocarpa* seeds which were used in producing flour respectively. Hence, it will provide information on the best cooking method for maximum protein retention in household and industrial application.

2. MATERIALS AND METHODS

2.1 Sourcing and Preparation of Materials

Sphenostylis sternocarpa (African yam bean) seeds used for this study was purchased from Ohafia and Umuahia in Abia State, Nigeria. The seeds used was a mixture of coloured cultivars of brown, red and white. They were winnowed, and extraneous materials were removed. The cleaned seeds were divided into four portions of 150 g each. After the removal of extraneous particles and cleaning of the beans, it was divided into four equal portions of 150 g each. One portion was left intact and untreated which served as control. Cooking practices included conventional cooking by adding water to the 150 g beans at 2:1 (w/w ratio) and boiling in an open pot for 120 minutes. Microwave cooking was completed by adding water to the 150 g beans at 2:1(w/w ratio) in a glass porcelain pottery and placing in a microwave (Sonic 5 mw-70017, Japan) for 810 minutes. Roasting of 150 g beans was completed in an oven preheated to 1500C for 20 minutes in the absence of moisture. The conventionally cooked and Microwave cooked preparations were dried at 65℃ in an oven (Ocean Med., Mode DHG- 9053A, England) for 6 h. The 4 bean preparations were ground electrically to fine flour to pass mesh sieves and saved in air tight containers for chemical analysis.

2.2 Nitrogen Determination

Nitrogen was determined was determined by the micro Kjedhal method descibed by [9]. 5 g of each sample was weighed and put in the Kjeldhal digestion flask and 10 ml concentrated sulphuric acid was added. 0.5 g Catalyst mixture (containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in the ratio of 10:5:1) was added into the flask to facilitate digestion. Four pieces of anti-bumping granules were added. The flask was then put in Kjeldhal digestion apparatus and heated for 3 hours until the liquid turned light green. Each sample digest was cooled and diluted with distilled water to 100 ml in standard volumetric flask. 10 ml of each diluted solution was mixed with 10 ml of 45% sodium hydroxide and put into Markham distillation apparatus and distilled into 10 ml of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70 ml of distillate was collected

respectively. Each sample distillate was then titrated with standardize 0.01 N hydrochloric acid to grey colored end point.

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

A = Titer value of the digested samples
B = Titer value of blank sample
V = Volume after dilution (100 ml)
W = Weight of dried sample (mg)
C = Aliquot of sample used (10 ml)
14 = Nitrogen constant in mg

2.3 Amino Acid Determination

Amino acid composition of the individual powder samples was determined by the method described by [10] using Ion Exchange chromatography (Technicon Sequential Multi-sample (TSM) amino acid analyser , Technicon Instruments Cooperation, New York, USA). Each flour sample was hydrolyzed. Hydrolysis was done by defatting 5 g of each flour sample using chloroform/ methanol mixture (2:1). Each defatted sample was put into glass ampoule respectively and subsequently, protein in each sample was hydrolyzed in 7 ml 6N HCl. Tryptophan was destroyed by this acid hydrolysis while oxidation of methionine and cysteine in the samples were prevented by passing nitrogen gas into the glass ampoule containing the hydrolyzed protein before sealing using Bunsen burner flame and put in an oven preset at 105℃± 50C for 2 hours. After this time, the ampoules were allowed to cool before broken open at the tip and the contents were filtered to remove the humins respectively. Each acid hydrolyzate was evaporated under vacuum and put into sample bottles respectively. pH was maintained by adding 5 ml acetate buffer (pH 2.0). 10 µl of each sample hydrolyzate was dispensed into the cartridge of the TSM amino acid analyser. Analysis lasted for 76 minutes. An integrator was attached to the Analyzer. This calculated the peak area proportional to the concentration of each amino acids. The net height of each peak produced by the chart record of TSM (each representing an amino acid) was measured and calculated. Norleucine was used as internal standard. Tryptophan was not determined. Amino acid values from the chromatogram peaks were calculated whereby the half height of each peak on the chart was found and width of the peak on the half height was accurately measured and recorded. Area of each peak was then obtained by multiplying the height by the width at the half height the Norleucine equivalent (NE) for each amino acid in the mixture.

$$NE = \frac{\text{Area of Norleucine Peak}}{\text{Area of Amino Acid}}$$

A constant 'S' was calculated for each amino acid in the standard mixture as:

$$Sstd = NEstd \times \text{molecular weight} \times \mu AAstd$$

The amount of each amino acid present in each sample was calculated in g/100g protein =
NH x W@ NH/2 x Sstd x C (Concentration of amino acid in aliquot of sample used)

$$\text{Where } C = \frac{\text{Dilution} \times 16}{\text{Sample Wt(g)} \times N\% \times 10 \times \text{Vol. Loaded}}$$

Where

NH = Net Height
W = Width at half height
Nleu = Norleucine

2.4 Estimation of Amino Acid Score and Predicted Protein Efficiency Ratio (P-PER)

Amino acid score of each flour sample was determined based on whole Hen's egg [11]. In this method, essential amino acids were scored methionine + cysteine, and phenylalanine + tyrosine was taken as two distinct units. Amino acid scores (AMSS) were estimated by [12] formula:

$$AMSS = \frac{\text{mg of amino acid/g of test protein} \times 100}{\text{mg of amino acid /g of reference protein}}$$

The predicted protein efficiency ratio (P-PER) was calculated from the amino acid composition using the equation developed by [13] stated thus:

P-PER = - 0.468 + 0.454 (Leu) - 0.105 (Tyr).

3. RESULTS AND DISCUSSION

The effect of different processing methods on amino acid composition of African yam bean (*Sphenostylis sternocarpa)* flour is shown in Table 1. Results revealed that glutamic acid had the highest concentration in both raw and processed samples with values 12.79, 11.30, 11.94, 12.58 g/100 g protein for the fresh, conventionally cooked, microwave cooked and roasted samples respectively. Methionine had the least concentration in all the flour samples

which ranged between 0.69 to 0.96 g/100 g protein. Roasting slightly increased methionine content while conventional and microwave cooking decreased it when compared to its value in the raw flour sample. The low methionine content in the various powder samples agrees with findings that methionine is the most limiting essential amino acid in leguminous seeds [14]. The most abundant essential amino acids were lysine and leucine which ranged between 5.88 to 6.82 g/100 g protein and 6.16 to 6.86 g/100 g protein respectively.

All the processing methods resulted in a decrease in lysine content of *Sphenostylis sternocarpa* flour while leucine content slightly increased to 6.86 g/100 g protein as a result of roasting with a slight decline being induced by conventional and microwave cooking. The reason could be as a consequence of the hydrophobicity of leucine which makes it to be located in hydrophobic regions of polypeptides and so may not be easily lost by roasting which does not involve a water medium. Nutrients most times get solubilized in water medium during processing resulting in a decrease. Lysine is the most sensitive amino acid and prone to processing damage [15]. A different observation was reported by [16] indicating that cooking and roasting resulted in a slight increase in both leucine and lysine content of black turtle bean. However, [17] reported that boiling led to a small decrease in lysine while leucine increased slightly in *Artocarpus heterophyllus* seeds. Other hydrophobic amino acids include isoleucine, valine, methionine, phenylalanine, tryptophan [18]. It was observed that these amino acids increased slightly by roasting. This could be as a result of the unfolding of the polypeptide chain when compared with the effect of both conventional and microwave cooking except for phenylalanine. Tryptophan was not determined. Lysine content of *Sphenostylis sternocarpa* flour from the raw seeds (6.82 g/100 g crude protein) was comparable to lysine content of raw *Phaseolus vulgaris* (black turtle bean) (6.50 g/100 g crude protein) [16] but higher than lysine content of cream coated Bambara nut (3.0 g/100 g crude protein), dark coated Bambara nut (2.9 g/100 g crude protein), cranberry beans (3.1 g/100 g crude protein), kresting's groundnut (3.0 g/100 g crude protein), brown coated cowpea (2.8 g/100 g crude protein), white coated cowpea (2.9 g/100 g crude protein) [19]. It entails that Sphenostylis sternocarpa is a good leguminous seed with appreciable lysine content needed in human nutrition. Lysine is essential for children as it is critical for bone

Table1. Effect of different processing methods on Amino acid profile of Sphenostylis sternocarpa seeds

Amino acid (g/100 g protein)	Raw	Conventionally cooked	Microwave cooked	Roasted	FAO/WHO (1991 Reference Pattern). (g/100 g protein)
Lysine*	6.82	5.88	6.65	6.49	5.80
Histidine*	3.49	3.05	3.43	2.98	2.80
Arginine*	5.44	4.93	5.44	5.44	5.20
Aspartic acid	8.71	7.51	7.89	8.90	7.70
Threonine*	3.63	3.06	3.29	4.09	3.40
Serine	4.20	3.46	3.82	4.23	7.00
Glutamic acid	12.79	11.30	11.94	12.58	14.70
Proline	3.59	3.01	3.01	4.17	10.70
Glycine	3.20	2.79	2.89	3.99	2.20
Alanine	3.44	2.81	2.98	3.89	6.10
Cyst(e)ine	1.52	1.24	1.31	1.31	3.00
Valine*	4.01	3.25	3.01	4.32	5.00
Methionine*	0.80	0.69	0.75	0.96	2.50
Isoleucine*	3.16	2.74	3.00	3.46	2.80
Leucine*	6.69	6.16	6.51	6.86	1.10
Tyrosine	2.98	2.65	2.81	3.14	1.10
Phenylalanine*	3.78	3.34	3.52	3.19	6.30
Tryptophan*	ND	ND	ND	ND	1.10
P-PER	2.26	2.05	2.19	2.31	-

**- Essential Amino Acid, ND – Not Determined, P-PER – Predicted Protein Efficiency Ratio*

formation, is involved in hormone production, lowers serum triglyceride levels [20]. Results indicated that lysine, histidine, glycine, isoleucine, leucine and tyrosine were sufficient to meet nutritional needs of man based on [12] reference pattern for amino acids in both raw and processed flour samples from *Sphenostylis sternocarpa* seeds. Microwave cooking and roasting did not cause any reduction in arginine in all the flour samples with a value of 5.44 g/100 g protein. Tyrosine is a non-essential aromatic amino acid. Predicted protein efficiency ratio (P-PER) is one quality parameter used for protein evaluation [12]. Results indicated roasting resulted to an increased P-PER with a value of 2.31 while conventional cooking and microwave cooking resulted in a decrease in P-PER with values of 2.05 and 2.19 respectively. P-PER of flour from raw seed samples of *Sphenostylis sternocarpa* was 2.26. P-PER of flour from raw, roasted, microwave cooked and conventionally cooked *Sphenostylis sternocarpa* seeds were slightly lower than P-PER of raw, cooked, boiled and roasted *Phaseolus vulgaris* [16] as well as raw, cooked and roasted groundnut [21] but higher than cooked and raw *Cyperus esculentus* seeds [22], cooked and raw *Artocarpus heterophyllus* seeds [17]. Much of proteins benefits may be attributed to leucine due to its ability to stimulate protein synthesis; helps turn on the body's switch to build muscle and spare muscle when dieting [23,24]. Protein sparing effects are primarily derived from leucine [24]. A protein efficiency ratio below 1.5 appropriately describes a protein of low or poor quality [25]. Leucine content above 5.0 g/100 g protein often results to appreciable P-PER [26]. It entails that roasted *Sphenostylis sternocarpa* seeds will be more beneficial in contributing proteins in human nutrition than conventionally and microwave cooked seeds.

Differences in amino acid concentration of flour from raw and processed *Sphenostylis sternocarpa* seeds is shown in Table 2. Data showed that the various processing methods caused a decrease in lysine, histidine, glutamic acid, cyst(e)ine and phenylalanine. The effect of these processing methods on amino acid decrease was in this order: Conventional cooking > microwave cooking > Roasting. Roasting resulted to enhancement of aspartic acid (2.18%), Threonine (12.67%), Serine (0.71%), proline (16.16%), glycine (24.69%), alanine (13.08%), valine (7.73%), methionine (20%), isoleucine (9.49%), Leucine (2.54%) and Tyrosine (5.37%). Conventional cooking and microwave cooking resulted in a decrease in all the amino acids except for arginine for flour from the microwave cooked seed samples. The drastic reduction in amino acids by conventional cooking more than microwave cooking could be as a result of much solubilization of amino acids in higher water volume employed in conventional cooking than in microwave cooking. Roasting is a dry heat cooking method. It enhanced the concentration of some amino acids which could be as a result of non-exposure to fluid-like environment whereby the unfolded polypeptide chains exposed the hydrophobic amino acids which were located in the interior. It made them more available, and the exposed amino acids were not solubilized in water medium employed in conventional cooking and microwave cooking.

Results on the different classes of amino acids are shown in Table 3. Total amino acids of flour from raw *Sphenostylis sternocarpa* seeds was 78.25 g/100 g crude protein. This value was comparable to total amino acids of *Phaseolus vulgaris* seeds (78.3 g/100 g crude protein) [16] but higher than what was reported for krestings groundnut (74.2 g/100 g crude protein), cream coated Bambara nut (70.8 g/100 g crude protein), dark brown coated Bambara nut (68.5g/100 g crude protein), cranberry beans (65.9g/100 g crude protein) [19]. Processing caused a change in total amino acids which varied in this order: Roasting > microwaved cooked > conventionally cooked with values of 80g/100g crude protein, 72.25 g/100 g crude protein and 67.87g/100 g crude protein respectively. The effect of roasting in enhancing amino acids of *Sphenostylis sternocarpa* is in agreement with the findings of [16] but differ for cooking which improved amino acid concentration.

The total non-essential amino acids (TNEAA) in flour for both raw and processed *Sphenostylis sternocarpa* indicated that flour from roasted seeds had the highest value of 42.21 g/100 g crude protein while flour from raw seeds had a value of 40.43 g/100 g. Total non-essential amino acids in flour from conventionally cooked seeds had the least value of 34.77 g/100 g crude protein. Total non-essential amino acids of flour from raw *Sphenostylis sternocarpa* was higher than what was reported for raw *Phaseolus vulgaris* (37.2 g/100 g crude protein) [16], cream coated Bambara nut (38.5 g/100 g crude protein), brown cowpea (36.19/100 g crude protein), white cowpea (35.4 g/100 g crude

protein), cranberry bean (34.1 g/100 g crude protein) [19]. However, TNEAA in flour from raw *Sphenostylis sternocarpa* seeds was comparable to that of krestings groundnut (41.4 g/100 g crude protein) and brown coated Bambara nut (40.10 g/100 g crude protein) [19].

Table 2. Results on Differences in Concentration of Various Amino Acid (g/100g crude protein) of flour from raw and processed *Sphenostylis. Sternocarpa* seeds

Amino acid (g/100 g protein)	Raw (i)	Conventionally cooked (i-ii)	Microwave cooked (i-iii)	Roasted (i-iv)	Mean	SD	CV (%)
Lysine*	6.82	0.94(13.78%)	0.17(2.49%)	0.33(4.83%)	6.46	0.41	6.35
Histidine*	3.49	0.44(12.61%)	0.06(1.72%)	0.51(14.61%)	3.24	0.26	8.02
Arginine*	5.44	0.51(9.38%)	0.00(0.00%)	0.00(0.00%)	5.31	0.26	4.90
Aspartic acid	8.71	1.20(13.78%)	0.82(9.41%)	-0.19(-2.18%)	8.25	0.66	8.00
Threonine*	3.63	0.57(15.70%)	0.34(9.37%)	-0.46(-12.67%)	3.52	0.45	12.78
Serine	4.20	0.74(17.62%)	0.38(9.05%)	-0.03(-0.71%)	3.93	0.36	9.16
Glutamic acid	12.79	1.49(11.65%)	0.85(6.65%)	0.21(1.64%)	12.15	0.67	5.51
Proline	3.59	0.58(16.16%)	0.58(16.16%)	-0.58(-16.16%)	3.45	0.56	16.23
Glycine	3.20	0.41(12.81%)	0.31(9.69%)	-0.79(-24.69%)	3.22	0.54	16.77
Alanine	3.44	0.63(18.31%)	0.46(13.37%)	-0.45(-13.08%)	3.28	0.49	14.94
Cyst(e)ine	1.52	0.28(18.42%)	0.21(13.82%)	0.21(13.82%)	1.35	0.12	8.89
Valine*	4.01	0.76(18.95%)	1.00(24.94%)	-0.31(-7.73%)	3.65	0.62	16.99
Methionine*	0.80	0.11(13.75%)	0.05(6.25%)	-0.16(-20.0%)	0.80	0.12	15.00
Isoleucine*	3.16	0.42(13.29%)	0.16(5.06%)	-0.30(-9.49%)	3.09	0.30	9.71
Leucine*	6.69	0.53(7.92%)	0.18(2.69%)	-0.17(-2.54%)	6.56	0.30	4.57
Tyrosine	2.98	0.33(11.07%)	0.17(5.70%)	-0.16%(5.37%)	2.90	0.21	7.24
Phenylalanine*	3.78	0.44(11.64%)	0.26%(6.88%)	0.59(15.61%)	3.46	0.25	7.23
Tryptophan*	ND	ND	ND	ND	-	-	-

(i): Amino acid concentration in flour from raw seed sample, (i-ii): Amino acid concentration in flour from raw seed sample - Amino acid concentration in flour from conventionally cooked seed sample, (i-iii): Amino acid concentration in flour from raw seed sample - Amino acid concentration in flour from microwave cooked seed sample, (i-iv): Amino acid concentration in flour from raw seed sample - Amino acid concentration in flour from roasted seed sample

Table 3. Evaluation of Amino acid classes of flour from raw and processed *Sphenostylis sternocarpa* seeds

Amino acid description	Raw	Conventionally cooked	Microwave cooked	Roasted
Total amino acids (TAA)g/100 g	78.25	67.87	72.25	80.00
Total non-essential amino acids (TNEAA)	40.43	34.77	36.65	42.21
%TNEAA	51.67	51.23	50.73	51.48
Total essential amino acids (TEAA)				
With histidine	37.82	33.1	35.60	37.79
Without Histidine	34.33	30.05	32.17	34.81
% TEAA				
With histidine	48.33	48.77	49.27	47.24
Without histidine	43.87	44.28	44.53	43.51
Essential aliphatic amino acid (EAAA)	17.49	15.21	15.81	18.73
%EAAA	22.35	22.41	21.88	23.41
Total neutral amino acids (TNAA)	41.00	35.20	36.90	43.61
%TNAA	52.39	51.86	51.07	54.51
Total acidic amino acids (TAAA)	21.50	18.81	19.83	21.48
%TAAA	27.48	27.72	27.45	26.85
Total basic amino acids (TBAA)	15.75	13.86	15.52	14.91
%TBAA	20.13	20.42	21.48	18.64
Total sulfur amino acids (TSAA)	2.32	1.93	2.06	2.26
% Cyst(e)ine in TSAA	65.52	64.25	63.59	57.71

Total essential amino acids (TEAA) with and without histidine in flour from raw and roasted seeds were quite comparable. Results indicated that processing had varying effects on essential amino acids in the various flour samples in this order: Roasting > Microwave > Conventional cooking. Total essential amino acids of flour from raw *Sphenostylis sternocarpa* seeds was lower than that of flour from *Phaseolus vulgaris* seeds with and without histidine [16], but higher than what was observed as total essential amino acids with and without histidine in flour made from raw seeds of cream and brown coated Bambara nut, krestings groundnut, cranberry beans, brown and white coated cowpea reported by [19]. Arginine is thought to be conditionally essential for children up to 5years old and the elderly 60 and up while histidine is essential for children up to 5 years of age [27].

Results indicated that roasting slightly enhanced essential aliphatic amino acids with a value of 18.73 g/100 which compared with flour produced from raw seed sample which had a value of 17.49 g/100 g crude protein. Conventional and microwave cooking caused a decrease in values of 15.21 and 15.81 g/100 g crude protein respectively. Aliphatic amino acids have a large hydrophobic side chain with the branched-chain amino acids (BCAAs) making up the bulk of it. Leucine, isoleucine are components of branched chain amino acids the other being valine. Their molecules are rigid, and their mutual hydrophobic interactions are important for correct folding of proteins as these chains tend to be located inside the protein molecule [6]. These BCAAs had values of 6.69, 3.16 and 4.01 g/100 g crude protein in flour made from raw seeds (Table 1). Processing caused different changes with roasting enhancing them while conventional cooking and microwave cooking slightly reduced them. BCAAs make up a high proportion of amino acids burned by the muscles as fuel with leucine being the most abundant of the three [23]. Leucine tends to modulate insulin signaling and glucose use by skeletal muscles through stimulation of glucose cycling via alanine cycle [24] while isoleucine induces glucose uptake by cells [28].

Total acidic amino acids (TAAAs) was higher than total basic amino acids (TBAAs) in flours made from both raw and processed *Sphenostylis sternocarpa* seeds. Variations in TAAAs in flour samples was in this order: Raw = Roasted > microwave cooked > conventionally cooked while differences in TBAAs in flours from the various samples was in this order Raw = microwave cooked > roasted >conventionally cooked.

Total sulphur amino acids (TSAAs) consists of the cyst(e)ine and methionine. It had values of 2.32 g/100 g crude protein in flour made from raw seeds while processing resulted in a slight decrease with values of 1.93, 2.06 and 2.26 g/100 g crude protein in flours made from conventionally cooked, microwave cooked and roasted *Sphenostylis sternocarpa* seeds respectively. TSAAs of flour from raw seeds was comparable to what was reported as TSAAs of *Phaseolus vulgaris*, Bambara nut, cowpea, cranberry beans, krestings groundnut [16,19], raw groundnut [21] but lower than TSAAs of flour from processed raw and cooked *Artocarpus heterophyllus* seeds which had values of 9.94 and 9.59 g/100 g crude protein respectively [17]. Sulphur amino acids provide sulphur for sulfating reactions in the body with cyst(e)ine having sparing effect for methionine [29]. Cyst(e)ine had a high percentage of TSAAs in flours from both raw and processed *Sphenostylis sternocarpa* seeds. Therefore implies that the available cyst(e)ine may have the potentials of sparing methionine which is an essential amino acid.

The essential amino acid scores (EAAS) based on provisional amino acid scoring pattern stated by [12] is shown in Table 4. Amino acid scores (AAS) indicated that flour from roasted seeds was sufficient in lysine, phenylalanine + tyrosine and threonine while flours made from raw, conventionally and microwave cooked seeds were sufficient in lysine and phenylalanine + tyrosine. Tyrosine is a non-essential amino acid but can spare phenylalanine [30]. It is a ring containing amino acid referred to as aromatic amino acid, the other two being phenylalanine and tryptophan with these being essential in human nutrition [6]. Phenylalanine has been reported to have anti-sickling potency [31].

The most limiting amino acids in both raw and processed flour samples were methionine + cyst(e)ine. Results also indicated that roasting slightly increased total amino acid scores (AAS) with a value of 6.95 while flour from raw seed sample had an AAS of 6.49. Microwave cooking had better effects on AAS than conventional cooking with values of 5.96 and 5.54 respectively. The most limiting amino acids were in this order: methionine + cysteine > valine > isoleucine in all the flour samples.

Table 4. Amino acid scores of flour from raw and processed *Sphenostylis sternocarpa* seeds

EAA	PAAESPAa (g/100 g protein)	RAW		Conventionally cooked		Microwave cooked		Roasted	
		EAAC	AAS	EAAC	AAS	EAAC	AAS	EAAC	AAS
Ile	4.0	3.16	0.79	2.74	0.69	3.00	0.75	3.46	0.87
Leu	7.0	6.69	0.96	6.16	0.88	6.51	0.93	6.86	0.98
Lys	5.5	6.82	1.24	5.88	1.07	6.65	1.21	6.49	1.18
Met + Cys (TSAA)	3.5	2.32	0.66	1.93	0.55	2.06	0.59	2.27	0.65
Phe+Tyr	6.0	6.76	1.13	5.99	1.00	6.33	1.06	6.33	1.06
Thr	4.0	3.63	0.91	3.06	0.77	3.29	0.82	4.09	1.02
Try	1.0	ND	ND	ND	ND	ND	ND	ND	ND
Val	5.0	4.01	0.80	3.25	0.65	3.01	0.60	4.32	0.86
Total	36	33.39	6.49	29.01	5.54	30.85	5.96	35.82	6.95

EAA – Essential amino acid, PAAESP – Provisional amino acid (egg) scoring pattern, EAAC – Essential amino acid concentration, AAS – Amino acid scores, ND – Not Determined , a[32]

4. CONCLUSION

It is therefore concluded that processing had different effects on the amino acid composition of flour from *Sphenostylis sternocarpa* seeds. All the essential amino acids were sufficient to meet human nutritional needs based on FAO/WHO [12] reference pattern for amino acids except valine, methionine and phenylalanine. Amino acid scores indicated that the most limiting amino acids were methionine + cysteine in both raw and processed flour samples while valine and isoleucine ranked second and third limiting amino acids in flour samples produced from raw and processed *S. sternocarpa* seeds. The present study indicated that total and particularly essential amino acids contents and amino acid composition of the flour samples changed by the processing methods. Among them, roasting enhanced the contents of amino acids in comparison to conventional cooking and microwave cooking. It is useful for the cooking in household and the industrial application.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ojiako OA, Igwe CU, Agha NC, Ogbuji CA, Onwuliri VA. Protein and amino acid composition of *Sphenostylis stenocarpa, Sesamum indicum, Monodora myristica,* and *Afzelia africana* seeds from Nigeria. Pakistan Journal of Nutrition. 2016;9(4): 368-372.
2. Adebowale OJ, Maliki K. Effect of fermentation period on the chemical composition and functional properties of pigeon pea (*Cajanus cajan*) seed flour. International Food Research Journal. 2011;18(4):1329-1333.
3. Adebowale KO, Lawal OS. Comparative study of the functional properties of Bambara groundnut (*Voandzeia subterranean*) Jack bean (*Cavnalia ensiformis*) and Mucuna bean (*Mucuna pruriens*). Food Research International. 2004;37:355-365.
4. Duranti M, Gius C. Legume seeds: Protein content and nutritional value. Field Crops Research. 1997;53:31-45.
5. Oloyo RA. Chemical and nutritional quality changes in germinating seeds of *Cajanus cajan* L. Food Chemistry. 2004;85:497-502.
6. Amaechi NC, Nwachukwu AN, Ubanwadi C. Amino acid composition and the effect of cooking on nutrient and functional properties of gastropod, *Viviparus intertextus*. Journal of Advances in Food Science and Technology. 2016;3(4):182–189.
7. Okigbo BN. Introducing the African yam bean (*Sphenostylis sternocarpa*). In processing. The first IITA Grain Legume Improvement workshop, IITA 29 Oct – 2 Nov 1973. Ibadan. 1993;224-238.
8. Nwosu JN, Onuegbu NC, Ogueke CC, Kabuo NO, Omeire GC. Acceptability of moin-moin produced from blends of African yam bean (*Sphenostylis stenocarpa*) and cowpea (*Vigna unguiculate*). International Journal of Current Microbiology and Applied Sciences. 2014;3(5):996-1004.

9. James CS. The analytical chemistry of foods. Blackie Academics and Professional, New-York; 1995.
10. Benitiez LV. Amino acid and fatty acid profile in aquaculture nutrition studies. In: Desilva SS (Ed). Fish nutrition research in Asia. Proceedings of third Asian Fish Nutrition Network meeting. Asian Fisheries Society, Manila, Philippines. 1989;23-25: 161.
11. Paul AD, Southgate AT, Russell J. First supplement to Melan and Widdowson's, the composition of foods. HMSO, London. 1976.
12. FAO/UN/WHO, Food and Agricultural Organization of the United Nations World Health Organization. Protein quality evaluation. Report of joint FAO/WHO expert consultation. FAO Food and Nutrition Paper, 51 (FAO) Rome. 1991;19-21:180.
13. Alsmeyer RH, Cunningham AE, Happich ML. Equation to predict protein efficiency ratio (PER) from amino acid analysis. Food Technology. 1974;28:34-38.
14. Laura S. Protein complementation. American Society for Nutrition Journals; 2011.
Available:www.nutrition.org/asnblog/2011/03/
(Accessed 31 August 2016)
15. Hurrell RF, Finot PA. Food processing and storage as a determinant of protein and amino acid availability. Experientia. 1983; 44:135-156.
16. Audu SS, Aremu MO, Lajide L. Influence of traditional processing methods on the nutritional composition of black turtle bean (*Phaseolus vulgaris* L.) grown in Nigeria. International Food Research Journal. 2013;20(6):3211-3220.
17. Amaechi NC, Oluagha NE. Effect of boiling on amino acid composition of jackfruit (*Artocarpus heterophyllus*) seeds from south-eastern Nigeria. Journal of Advances in Food Science and Technology. 2016;3(4):175-181.
18. Betts MJ, Russell RB. Amino acid properties and substitution. In: Barnes, M.R. and Gray, C.C (Eds.). Bioinformatics for Genetics. Wiley Publishers; 2003.
19. Aremu MO, Olaofe O, Akintayo ET. A comparative study on the chemical and amino acid composition of some Nigerian under-utilized legume-flours. Pakistan Journal of Nutrition. 2006;5(1):34-38.
20. Gersten D. The 20 amino acids: What they are and how they keep you alive and vibrant. The Gersten Institute for Higher Medicine, San Diego, CA, USA; 2013.
21. Adeyeye EI. Effect of cooking and roasting on the amino acid composition of raw groundnut (*Arachis hypogaea*) seeds. Acta Sci. Pol. Technol. Aliment. 2010;9(2):201-216.
22. Aremu MO, Bamidele TO, Agere H, Ibrahim H, Aremu SO. Proximate composition and amino acid profile of raw and cooked black variety of tiger nut (*Cyperus esculentus* L.) grown in north-east Nigeria. Journal of Biology Agriculture and Healthcare. 2015;5(7):213-221.
23. Volek JS. How much do you need of this branched chain amino to get results? Health and wellness news; 2006.
Available:www.Nutritionexpress.com
24. Layman DK, Walker DA. Potential importance of leucine in treatment of obesity and the metabolic syndrome. Journal of Nutrition. 2006;136(1):319S–323S.
25. Friedman MC. Nutritional value of protein from different food sources: A review. Journal of Agriculture and Food Chemistry. 1996;44(1):6-29.
26. Amaechi NC, Njoku B, Adiele AM. Evaluation of the amino acid profile of *Cnidoscolus acontifolius* and *Ceiba pentandra* leaves grown in south-east Nigeria. International Journal of Applied Research and Technology. 2015;4(6):88–95.
27. Sowers S. A primer on branched chain amino acids. Smart supplementation, literature education series on dietary supplements. Huntington College of Health Sciences; 2009.
28. Doi M, Yamaoka T, Nakayama M, Sugahara K, Yoshizawa F. Hypoglycemic effect of isoleucine involves increased muscle glucose uptake and whole body glucose oxidation and decreased hepatic gluconeogenesis. American Journal of Physiology-Endocrinology and Metabolism. 2007;292(6):E1683-93.
29. Fukagawa NK, Yu YM, Young VR. Methionine and cysteine kinetics at different intakes of methionine and cysteine in elderly men and women. Journal of Clinical Nutrition. 1998;68:380-388.
30. Pencharz PB, Hsu JW, Ball RO. Aromatic amino acid requirement in healthy human

subjects. Journal of Nutrition. 2007; 137(6 suppl.1):15765-15785.

31. Ekeke GI, Shode FO. Phenylalanine is the predominant antisickling agent in *Cajanus cajan* seed extract. Planta Medica. 1990; 56(1):41–43.

32. Belschant AA, Lyon CK, Kohler GO. Sunflower, safflower, sesame and castor protein. In: Pine, N.W (Ed) Food protein sources, University Press, Cambridge UK. 1975;79-104.

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