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Compositional Evaluation of Raw and Processed Harms (*Brachystegia Eurycoma*) Seed Flour

Aremu, M. O.[1*], Ohale, I. M.[2], Magomya, A. M.[1], Longbap, D. B.[1], Ushie, O. A.[1]

1- Department of Chemical Sciences, Federal University Wukari, PMB 1020 Taraba State, Nigeria. 2- Department of Chemistry, Nasarawa State University, PMB 1022, Keffi, Nigeria.

Abstract

Brachystegia eurycoma is predominantly grown in the eastern region of Nigeria. Its seed is used in food majorly as a soup condiment, flavoring agent and soup thickener. The proximate, mineral and amino acid compositions were determined on raw and processed seeds (boiled, fermented and roasted) of *Brachystegia eurycoma* using standard analytical techniques. The processing methods showed deviations in the nutrients from the raw seeds. Crude protein was reduced by the processing methods with exception of the roasting method. Crude fat was reduced in all processing methods in this order: Raw>fermented>roasted>boiled samples. All of the processing methods enhanced calcium content in this order: Raw<boiled<fermented<roasted samples. Boiling and fermenting reduced the content of magnesium by 72.6 and 30.2%, respectively while boiling, fermenting and roasting increased potassium content by 124.5, 12.2 and 120.4%, respectively. Generally, all of the samples were found to be a good source of essential minerals, and harmful heavy metals such as lead, chromium, arsenic and cadmium were not at detectable range of atomic absorption spectrophotometer. The amino acid profile revealed that amino acids in the fermented sample were better concentrated than those in the raw and other processed samples. The total essential amino acids (TEAA) ranged from 28.63 g/100g crude protein in the roasted sample to 34.79 g/100g crude protein in the fermented sample. The limiting amino acid (LAA) was Met + Cys (TSAA) for all of the samples.

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Correspondence to
Aremu, M.O. Department of Chemical Sciences, Federal University Wukari, PMB 1020 Taraba State, Nigeria.
Email:
lekearemu@gmail.com

1. Introduction

Legumes are characterized to contain a high content of protein, fibre and micronutrients. The protein content of legumes ranges between 20–30% of energy which corresponds to the levels found in fish and meat [1]. Fundamental changes in the food system may be needed to increase the demand for legumes and other food with high nutritional value and low environmental impact [2]. The common benefits of legumes for overall health and environment suggest that legumes will have an important role in the development towards sustainable food consumption and pr-oduction. Exploring wild underutilized legumes would be of great significance for food security meeting the nutritional requirement and contributing to the improvement of nations' economy. Legume seeds are widely distributed, and grown for their nutritious seed in parts of the western and eastern Africa [3, 4]. They can be milled into flour, and used to make bread, doughnuts, chips and infant formula [5]. They are used industrially to produce biodegradable plastics, oils, gums, dyes and inks [6]. Many legumes have applications in folk medicine [7]. The seeds possess certain bioactive compounds with health promoting or therapeutic value. The presence of polyphenolic compounds in legumes is beneficial as natural polyphenols have been reported to exert potential health benefits in the prevention of cardiovascular diseases; this is mainly due to their antioxidant capacity. They can interact with and scavenge the free radicals, and active antioxidant enzymes [8]. Legumes have been reported to be rich in essential amino acids, polyunsaturated fatty acids, and dietary fibre, along with beneficial compounds [9–11].

Brachystegia eurycoma is called "achi" by the Igbo tribe in Nigeria. It is a huge tree with twisted, spreading branches and the bark often extruding buttery gum. It is cultivated in the south–eastern Nigeria, and used for soup as thickener. The seeds are found abundantly and underutilized; therefore, they can be explored commercially for their nutritional value, and their oils might provide extra income for local population [12].

The effect of processing on the nutritional components of legumes gives better results in human diet. The presence of relatively high concentration of toxins such as lecithin, tannin, oxalate and phytate affects legumes' nutritional quality by interacting with the intestinal tract, and reducing protein digestibility and amino acid absorption [13]. Some researchers [14, 15] have shown that our traditional processing techniques such as cooking, roasting, boiling and fermenting could reduce or eliminate the anti–nutritional factors of foods. The objective of the present study was to assess the efficiency of processing methods such as boiling, fermenting and roasting for changing the chemical composition of *B. eurycoma* seed flour with a view to providing preliminary information towards effective utilization of this seed in various food applications in Africa.

2. Materials and Methods

2.1 Collection of samples

The seeds of *B. eurycoma* were purchased from Eke–Ata market in Imo State, Nigeria. Then they were thoroughly separated from impurities while the cleaned seed samples were wrapped in polythene bag, sealed in air– tight plastic container, and stored at room temperature prior to application of different processing methods.

2.2 Preparation of samples

2.2.1 Raw seeds

The seeds were dehulled by gent roasting for 5 min and then soaking in tap water for 3 h.

2.2.2 Boiled seeds

Four hundred grams of dehulled seeds were boiled for 45 min in distilled water at 100°C. The boiled seeds were drained using a perforated basket, and then dried in an oven at 50°C.

2.2.3 Fermented seeds

The dehulled seeds (400 g) were wrapped in blanched banana leaves and allowed to ferment for 3 days according to the method described by [16]. The sample was then removed, dried in an oven at a temperature of 50°C.

2.2.4 Roasted seeds

The remaining portion (400 g) of the dehulled seeds was roasted in a hot iron pan until the seeds turned from green to brown. The roasted seeds were later dried at 50^{o}C until a constant weight was obtained.

Each processed sample was then ground into fine powder with a food blender. The powdered samples were stored in air tight containers prior to analyses.

2.3 Proximate analysis

The moisture, ash, crude fat, crude fiber, crude protein (N×6.25) and carbohydrate (by difference) were determined in accordance with the methods of [17]. All proximate analyses of the legume flours were carried out in triplicate and reported in percent. All chemicals were of analytical grade.

2.4 Mineral analysis

All of the metals were determined by Atomic Absorption Spectrophotometer (Solar 969 Unicam) with the exception of sodium and potassium, which were determined using a flame photometer (Model 405, Corning, UK).

2.5 Amino acid analysis

The amino acid analysis was by Ion Exchange Chromatography [18] using the Technicon Sequential Multisample (TSM) amino acid analyzer (Technicon Instruments Corporation, New York). The period of analysis was 76 min for each sample. The gas flow rate was 0.50 ml/min at 60^{o}C with reproducibility consistent within ± 3 min. The net height of each peak produced by the chart recorder of the TSM (each representing an amino acid) was measured and calculated. The amino acid values reported were the averages of two determinations. Nor–leucine was the internal standard. Tryptophan was not determined.

2.6 Determination of isoelectric point (pI), quality of dietary protein and predicted protein efficiency ratio (P–PER)

The predicted isoelectric point was evaluated according to [19] as below:

$$\text{pIm} = \sum_{i=1}^{n=1} pI_i X_i$$

where, pIm = the isoelectric point of the mixture of amino acids. pI_i = The isoelectric point of the i^{th} amino acids in the mixture. X_i = the mass or mole fraction of the amino acids in the mixture.

The quality of dietary protein was measured by finding the ratio of available amino acids in the sample protein compared with the needs expressed as a ratio. Amino acid score (AAS) was then estimated by applying the [18] formula:

$$\text{AAS} = \frac{mg\ of\ amino\ acid\ in\ 1g\ of\ test\ protein}{mg\ of\ amino\ acid\ in\ 1g\ reference\ protein}\ x\ \frac{100}{1}$$

The Predicted Protein Efficiency Ratio (P–PER) of the seed sample was calculated from their amino acid composition based on [20] equation:

$$\text{P–PER} = –0.468 + 0.454\ (\text{Leu}) –0.105\ (\text{Tyr})$$

2.7 Statistical analysis of the samples

The energy values were calculated by adding up the carbohydrate x 17 kJ, crude protein x 17kJ and crude fat x 37 kJ for each of the samples. Errors of three determinations were computed as standard deviation (SD) for the proximate composition. The grand mean, SD, and coefficient variation (CV %) were also determined.

3. Results and Discussion

The mean proximate composition of raw and processed *B. eurycoma* is presented in Table 1. The crude protein values of *B. eurycoma* ranged from 20.17 to 25.83%. The crude protein is higher when compared with earlier reports. A range of 3.25– 12.77% of crude protein was obtained for the raw sample of this seed by some workers [16, 21, 22], which is lower than the result obtained in this study. *D. microcarpum,* a type of hydrocolloid seed, gave 37.1% crude protein [23]. The protein content of the seed can be considered well comparable with some protein- rich legumes such as cranberry beans, pigeon peas and soy bean [24, 25]. *B. eurycoma* contains crude lipid content in the range of 14.09–15.45%. These values are comparable with the earlier reports on the seed, which ranged from 10.52 to 29.00% fat [16, 26].

The percentage fat of *D. microcarpum,* a type of hydrocholloid, is significantly higher than value obtained in the present study. The difference in the fat content may be attributed to the type of species or the duration of storage of the seed (shelf-life) [16].

Crude fibre in the diet consists mostly of the plant polysaccharides that cannot be digested by human dietary enzymes such as cellulose, hemicelluloses and some materials that make up the cell wall [27]. It is a significant component in the body. It increases stool bulk, and decreases the time that waste materials spend in the gastro-intestinal tracts. The fibre content values obtained in the raw and processed powdered samples of *B. eurycoma* ranged from 3.42 to 5.25%. These values are in agreement with the earlier reported values on *B. eurycoma* seed (2.2–4.35 %) [16,26].

Table 1. Mean proximate composition (%) of raw and processed *B. eurycoma* seed flour

Parameter	Raw	Boiled	Fermented	Roasted	Mean	SD	CV%
Crude protein	23.73 ± 0.20	22.32 ± 0.11	20.17 ± 0.02	25.83 ± 0.05	23.01	2.15	14.54
Crude fat	15.45 ± 0.10	14.14 ± 0.05	14.09 ± 0.10	15.26 ±0.04	14.74	0.34	5.32
Crude fibre	5.25 ± 0.13	3.42 ± 0.12	3.76 ± 0.30	3.55 ± 0.13	4.00	2.79	3.84
Moisture	6.14 ± 0.03	5.81 ± 0.01	4.32 ± 0.05	5.29 ± 0.20	5.39	0.69	12.80
Ash	7.19 ± 0.03	6.29 ±0.01	7.18 ± 0.03	6.17 ± 0.05	6.71	0.48	7.15
Carbohydrate	50.26 ± 0.20	50.22 ± 0.03	52.31 ± 0.20	51.26 ± 0.20	51.03	1.05	2.31
Energy (KJ/100g)	1390.35	1401.02	1399.34	1826.28	1504.25	185	12.29
Fatty acids	8.36	7.31	7.27	8.21	7.80	0.50	6.40

Calculated metabolizable energy (kJ 100/g) (protein x 17 ± fat x 37 ± carbohydrate x 17); calculated fatty acid (0.8 x crude fat)

Table 2. Difference in mean proximate composition (%) of raw and processed *Brachystigia eurycoma* seed flour

Parameter	I-II	I-III	I-IV	Mean	SD	CV (%)
Protein	1.41(10.3%)	3.56(25.9%)	-2.1(-15.3%)	2.36	0.89	37.00
Crude Fat	1.31(12.5%)	1.36(13%)	0.19(1.8%)	0.95	0.54	56.80
Crude Fibre	1.83(34.86%)	1.09(28.38%)	1.70(32.38%)	1.54	0.42	2.35
Moisture	0.33(5.4%)	1.82(29.6%)	0.85(13.8%)	1.00	0.62	62.00
Ash	0.9(12.5%)	0.01(0.14%)	1.02(14.2%)	0.64	0.45	70.30
Carbohydrate	-0.01(-0.00%)	-2.05(-0.04%)	-1.00(-0.02%)	1.02	0.25	60.52
Energy kJ/100g	-10.67(-0.7%)	-8.99(-0.6%)	-435(–0.31%)	151.6	141	93.00
Fatty Acid	1.05(12.5%)	1.09(13.0%)	0.15(1.7%)	0.76	0.20	380.00

I = Raw; II = Boiled; III = Fermented; IV = Roasted; SD = Standard deviation; CV% = Coefficient of variation

The value of moisture content obtained ranged from 4.32% to 6.14% in the raw and processed samples, which is within the expected range for most of *B. eurycoma* seeds [16]. The fermented sample contained less moisture than the raw and other processed samples, which can be attributed to the activities of microorganism during the fermentation. This result is comparable with the finding of a study on the effect of fermentation on the nutritive value of *B. eurycoma*, which reported the moisture content of unfermented and fermented samples as 6.19% and 5.95%, respectively. When compared with other legumes' moisture content values in the literature, this value is on the high side [9, 11]. The ash content of the seed ranged from 6.17 to 7.9% in the raw sample. This result is higher when compared with the results (1.48-4.5%) obtained on *B. eurycoma* [16]. The ash content is equally high when compared with other legumes such as kersting groundnut, cowpea and cranberry beans (3.2–4.6%) [24]. But, it is comparable with the total ash obtained for *Tilapia quinnesis* (8.2%) [28]. There was a decrease on the ash value in the boiled and roasted samples.

Carbohydrate content of the seed ranged from 41.29 to 52.36%. The fermented sample had the highest value of carbohydrate though it is low when compared with *Rhynchosia* species (64.25–72.51%) [29]. The fermented sample is comparable with the result obtained by [16], likely due to the fact that during the fermentation, carbohydrate (glucose) is converted into CO_2, H_2O and heat. This appears that fermented sample of *B. eurycoma* can be a better source of carbohydrate.

The fatty acid and calculated metabolizable energy values of *B. eurycoma* were in the range of 7.27-8.36% and 1390.35–1826.28 kJ/100g, respectively. The metabolizable energy of *B. eurycoma* is more favourable than that of cereals. Metabolizable energy is the energy that is readily available for the production of adinosine triphoshate (ATP).

Table 3. Mean mineral composition (mg/100g) of raw and processed *B. eurycoma* seed flour

Mineral	Raw	Boiled	Fermented	Roasted	Mean	SD	CV
Sodium (Na)	4.3	4.1	5.05	10.65	6.03	2.69	44.61
Potassium(K)	2.45	5.5	2.75	5.4	4.03	1.43	35.48
Magnesium (Mg)	10.75	2.95	7.5	12.75	8.49	3.71	43.70
Iron (Fe)	2.95	0.45	0.75	0.5	1.16	1.04	89.66
Zinc (Zn)	0.275	0.07	0.725	0.555	0.41	0.25	60.98
Copper (Cu)	0.97	1.02	1.075	1.06	1.03	0.041	3.98
Lead (Pb)	ND	ND	ND	ND	ND	ND	ND
Arsenic (As)	ND	ND	ND	ND	ND	ND	ND
Chromium(Cr)	ND	ND	ND	ND	ND	ND	ND
Cadmium (Cd)	ND	ND	ND	ND	ND	ND	ND
Manganese (Mn)	1.2	1.65	1.55	0.8	1.30	0.33	25.38
Calcium (Ca)	7.3	12.5	15.55	20.75	14.03	4.88	34.78
Phosphorus (P)	0.6	0.8	0.8	2.1	1.08	0.60	55.56
Ca/P	12.2	15.6	19.4	9.9	14.28	3.59	25.14
Na/K	1.76	0.75	1.84	1.97	1.58	0.48	30.38

Ca/P = Calcium/Phosphorus Ratio; Na/K = Sodium/Potassium Ratio; ND = Not detected

Table 4. Differences in the mean mineral composition of raw and processed *B. eurycoma* seed flour

Mineral	I – II	I – III	I – IV	Mean	SD	CV (%)
Na	0.2(4.7%)	-0.75(-17.4%)	-6.35(-147.7%)	2.43	2.78	114.40
K	-3.05(-124.5%)	-0.3(-12.2%)	-2.95(-120.4%)	2.10	1.27	60.50
Mg	7.8(72.6%)	3.25(30.2%)	-2(–18.6%)	4.35	2.49	57.20
Fe	2.5(84.7%)	2.2(74.6%)	2.45(83.1%)	2.38	0.13	4.46
Zn	0.21(74.5%)	0.45(-1.64%)	-0.28(-1.01%)	0.31	0.10	32.30
Cu	-0.05(-5.2%)	-0.11(-0.11)	-0.09(-9.3%)	0.083	0.025	30.10
Pb	ND	ND	ND	ND	ND	ND
As	ND	ND	ND	ND	ND	ND
Cr	ND	ND	ND	ND	ND	ND
Cd	ND	ND	ND	ND	ND	ND
Mn	-0.45(-37.5%)	-0.35(–29.2)	0.4(33.3%)	0.4	0.04	10.00
Ca	-5.2(-71.2%)	-8.25(-113%)	-13.45(–184.2%)	8.97	3.41	35.00
P	-0.2(-33.3%)	-0.2(-33.3%)	-1.5(-250%)	4.30	0.61	96.80
Ca/P	-3.4(-27.8%)	-7.2(-59%)	2.3(18.9%)	4.30	2.1	48.80
Na/K	1.01(57.4%)	-0.08(-4.5%)	-0.21(-11.9%)	0.43	0.41	95.30

I = Raw; II = Boiled; III = Fermented; IV = Roasted; ND = Not determined

It is utilized during the metabolic processes associated with digestion, absorption and inter-mediary metabolism of food. The fatty acid values obtained in the present study suggest that oils may be suitable for industrial purposes.

Table 2 displays the difference in the mean proximate composition of raw and processed *B. eurycoma*. The fat, crude fibre, moisture and ash contents recorded a decrease while carbohydrate showed an increase in all of the processing methods. The roasted sample showed an increase in crude protein by 15.3% while the boiled and fermented samples recorded a decrease by 10.3% and 25.9%, respectively; this reduction may be due to leaching [14]. The metabolizable energy was enhanced by all of the processing methods. The CV% ranged from 1.37 in crude fat to 380.00% in the calculated fatty acid.

The mineral composition of *B. eurycoma* is presented in Table 3. The results showed that the most abundant mineral in the

Table 5. Amino acid composition (g/100g crude protein) of raw and processed *B. eurycoma* seed flour

Amino	Raw I	Boiled II	Fermented III	Roasted IV	Mean	SD	CV %
Lysine [a]	3.46	3.24	3.40	3.03	3.28	0.17	5.18
Histidine [a]	2.27	2.18	2.37	2.02	2.21	0.13	5.88
Arginine [a]	4.40	4.14	4.57	3.88	4.25	0.26	6.12
Aspartic acid	12.16	11.51	11.91	10.52	11.53	0.62	5.38
Threonine [a]	3.12	3.39	3.20	2.87	3.15	0.19	6.03
Serine	2.60	2.31	2.63	2.09	2.41	0.22	9.13
Glutamic acid	9.70	9.39	10.07	8.33	9.37	0.65	6.94
Praline	3.46	3.15	3.46	2.65	3.18	0.33	10.38
Glycine	3.39	3.41	3.39	3.00	3.30	0.17	5.15
Alanine	4.09	3.99	4.18	3.80	4.02	0.14	3.48
Cystine	0.86	0.79	0.86	0.66	0.79	0.082	10.38
Valine [a]	3.42	3.24	3.45	2.75	3.22	0.28	8.70
Methionine[a]	0.91	0.91	0.89	0.65	0.84	0.11	13.10
Isoleucine [a]	3.01	2.76	3.52	2.70	3.00	0.32	10.67
Leucine [a]	8.25	8.00	9.26	7.70	8.30	0.59	7.11
Tyrosine	3.18	2.86	3.65	2.86	3.14	0.32	10.19
Phenylanine [a]	3.80	3.37	4.13	3.03	3.58	0.42	11.73
Tryptophan	ND	ND	ND	ND	-	-	-
pI	4.04	3.72	4.20	3.50	3.87	0.27	6.98
P–PER	2.90	2.90	3.40	2.70	2.98	0.26	8.72

I =Raw; II = Boiled; III = Fermented; IV = Roasted; a = [a]Essential amino acid; pI=Calculated Isoelectric point; P–PER = Predicted protein efficiency; ND = Not determined; SD=Standard deviation; CV = Coefficient of variation.

Table 6. Difference in the amino acid composition (g/100g crude protein) of raw and processed *Brachyslegia eurycoma* seed flour

Amino acid	I–II	I–III	I–III	Means	SD	CV%
Lysine [a]	0.22(6.36%)	0.06(1.73%	0.43(12.43%)	0.24	0.15	62.5
Histidine [a]	0.09(3.96%)	-0.1(-4.40%)	0.25(11.82%)	0.15	0.07	46
Arginine [a]	0.26(5.91%)	-0.17(–3.86%)	0.52(13.5%)	0.32	0.18	56.3
Aspartic Acid	0.65(5.35%)	0.19(1.56%)	1.64(8.01%)	0.83	0.59	71
Threonine [a]	-0.27(-8.65)	-0.08(-2.56%)	0.25(19.6%)	0.2	0.09	45
Serine	0.29(11-15%)	-0.03(–1.15%)	0.51(14.12%)	0.28	0.19	67.9
Glutamic acid	0.31(3.19%)	0.37(3.81%)	1.37(23.4%)	0.68	0.47	69.1
Proline	0.31(8.96%)	0.0(0.00)	0.81(11.50%)	0.56	0.25	44.6
Glycine	-0.02(-0.58%)	0(00)	0.39(7.09%)	0.21	0.19	90.5
Alanine	0.1(0.024%)	-0.09(-2.20%)	0.29(23.26%)	0.16	0.09	56.3
Cystine	0.07(8.14%)	0(0)	0.2(19.59%)	0.14	0.04	28.6
Valine [a]	0.18(5.26%)	-0.03(–0.88%)	0.67(28.57%)	0.29	0.27	93.1
Methionine[a]	0(000)	0.02(2.19%)	0.26(10.57%)	0.14	0.12	85.7
Isoeucine [a]	0.22(7.3%)	-0.51(-16.9%)	0.31(10.29%)	0.23	0.17	73.9
Leucine [a]	0.25(3.03%)	-1.01(-12.2%)	0.55(6.67%)	0.60	0.31	51.7
Tyrosine	0.32(10.06%)	-0.47(-14.78%)	0.32(10.6%)	0.37	0.07	18.9
Phenylanine [a]	0.43(11.32%)	–0.33(-8.68)	0.77(20%)	0.51	0.19	37.2
Tryphophan	ND	ND	ND	-	-	-
pI	0.32(7.92%)	-0.16(-3.96%)	0.54(13.37%)	0.34	0.16	47.0
P-PER	0(000)	-0.5(-17.24%)	0.7(6.89%)	0.35	0.2	57.1

I = Raw Sample; II = Boiled Sample; III = Fermented Sample; IV = Roasted Sample;
a = [a]Essential amino acid; pI =Calculated isoelectric point; P-PER = Predicted protein efficiency;
ND = Not detected; SD = Standard deviation; CV% = Coefficient of variation.

raw sample is magnesium (10.75 mg/100 g) followed by calcium (7.3 mg/100g), sodium (4.3 mg/100g), potassium (2.45 mg/100g) while the least is zinc (0.275 mg/100g). These values are higher than those reported for *B. eurycoma* [16]. These variations could be as a result of the species, location and soil constituent of where the tree was planted [16]. The processed seed was found to possess higher calcium content than the raw (7.3 mg/100 g); boiled (12.5 mg/100g), fermented (15.55 mg/100g) and roasted (20.75 mg/100g) samples. This proves that the processing methods improved the calcium content of the seed. The magnesium content was reduced to 2.95 mg/100g when boiled, and to 7.5 mg/100g when fermented but increased to 12.75 mg/100g when roasted. This agrees with the results obtained by [14]. The sodium content increased from 4.3 mg/100g for the raw sample to 10.65 mg/100g for the roasted sample. There was an increase of the potassium content in the boiled and roasted samples of the seed by 124.5% and 120.4%, respectively. The reduction of the minerals by different processing methods can be attributed to leaching and metabolic activities [10]. *B. eurycoma* could serve as a good source of calcium, which is necessary for development of strong bones and teeth. Normal extra-cellular calcium concentrations are necessary for blood coagulation and for the integrity of intracellular cement substances. The use of this seed (*B. eurycoma)* can assist in supplementation of calcium for children with calcium deficiency. Magnesium works as co-factor for over 300 enzymatic reactions in the body. It is also required for all reactions involving ATP (Adinosine Triphosphate). ATP supplies the energy required for physical activity by releasing the energy stored in phosphate bonds. Report says that magnesium may help to support mineral bone density in elderly women and men [30]. Phosphorous assists calcium in many body reactions; modern diets that are rich in phosphorous may promote the loss of calcium to phosphorus ratio. This has led to the concept of "calcium to phosphorus ratio" (Ca:P). In the present study, we found the Ca:P of *B. eurycoma* to be in the range of 9.9-
19.4. Food is considered good if the Ca:P ratio is above 1 and poor if it is less than 0.5 while a Ca:P ratio above 2 helps to increase the absorption of calcium in the small intestine. The sodium to potassium ratio (Na:K) was found to be 0.75 to 1.97 in the seed. The Na:K ratio in the body is of great concern for the prevention of high blood pressure. The Na:K ratio less than 1 is recommended. Hence, boiled *B. eurycoma* would probably reduce high blood pressure disease because it had Na:K ratio less than one. With respect of the micro-nutrients, the values of copper and manganese were enhanced by boiling and fermentation, while boiling, fermentation and roasting reduced the values of iron from 2.95 to 0.45 mg/100g. This could be attributed to the effect of heat, changing the insoluble chemical

species of some trace elements into soluble ones, which were extracted into the water [31]. The CV% levels were highly varied with the highest variability recorded in iron (89.66%).

Table 4 shows the difference in mineral content between the raw and boiled, raw and fermented and raw and roasted samples of *B. eurycoma*. The processing methods increased the mineral contents of phosphorus, calcium, copper and potassium. Magnesium was increased by 18.6% in the roasted sample but was reduced by 72.6% and 30.2% in the boiled and fermented samples, respectively. Fermentation and roasting increased the sodium and zinc content, as well as the Na/K ratio. Manganese content and Ca/P ratio were increased by the boiling and fermentation processes. Iron was reduced by all of the processing methods employed. Lead, chromium, arsenic and chromium in the samples were not at detectable range of AAS, indicating that *B. eurycoma* seeds were free from toxic and undesirable minerals, which are harmful to the body system. The CV% levels ranged from 10% in manganese to 114.4% in sodium.

Table 5 shows the amino acid profile of *B. eurycoma*. Aspartic and glutamic acids were found to be the most abundant as expected in legumes. Aspartic and glutamic acids made up 21.86, 20.09, 21.90 and 18.85 g/100g crude protein in the raw, boiled, fermented and roasted samples, respectively. Leucine was the most concentrated (7.70-9.26 g/100g cp). For leucine and glutamic acid, the order of concentrations was as fermented > raw > boiled > roasted samples. The calculated isoelectric point (pI) ranged from 3.50 to 4.04. This is useful in predicting the pI for protein isolate from biological samples [19]. The predicted protein efficiency ratio (P – PER) is one of the quality parameters used for protein evaluation [32]. As shown in Table 5, the P-PER values ranged from 2.7 to 3.4, which are higher than the reported values on some legume crops (1.62) [33], and [34], but comparable with *Kerstingella geocarpa* and *Phaseolus coccineus* protein concentrates (2.24-2.36) [35].

Table 7. Amino acid classification (g/100 g crude protein) of raw and processed *B. eurycoma* seed flour

Parameter	Raw	Boiled	Fermented	Roasted	Mean	S D	CV%
Total amino acid (TAA)	72.08	68.67	74.94	62.52	69.55	4.63	6.66
Total essential amino acid (TEAA)							
With histidine	32.64	31.26	34.79	28.63	31.83	2.24	7.04
Without histidine	30.37	29.08	32.42	26.61	29.62	2.11	7.12
% TEAA							
With histidine	45.30	45.50	46.40	45.80	45.75	0.42	0.92
Without histidine	42.10	42.30	43.30	42.60	42.58	0.91	2.14
Total non-essential amino acid (TNEAA)	39.44	37.41	40.15	33.91	37.73	2.39	6.33
% TNEAA	54.70	54.50	53.60	54.22	54.26	0.41	0.76
Essential aliphatic amino acid (EAAA)	17.80	17.42	19.43	16.02	17.67	1.21	6.85
Essential aromatic amino acid (EArAA)	3.80	3.37	4.13	3.03	3.58	0.84	23.46
Total neutral amino acid (TNAA)	36.91	38.21	42.62	34.76	38.13	2.87	7.53
% TNAA	51.20	55.60	56.90	55.60	54.83	2.16	3.94
Total acidic amino acid (TAAA)	21.86	20.09	21.98	18.85	20.70	1.30	6.28
% TAAA	30.30	30.40	29.30	30.10	30.03	0.43	1.43
Total basic amino acid (TBAA)	10.13	9.59	10.34	8.93	9.75	1.09	11.18
% TBAA	14.10	13.90	13.80	14.20	14.00	0.32	2.29
Total sulphur amino acid (TSAA)	1.77	1.70	1.75	1.31	1.63	0.38	23.31
% cystine in TSAA	48.60	46.50	49.10	50.40	48.65	1.40	2.88

SD = Standard deviation; CV = Coefficient of variation

Table 8. Amino acid scores of raw and processed *B. eurycoma* seed flour

EAA	PAAESP (g/100g protein)	Raw EAAC	AAS	Boiled EAAC	AAS	Fermented EAAC	AAS	Roasted EAAC	AAS
Ile	4.0	3.01	0.8	2.79	0.7	3.52	0.9	2.70	0.7
Leu	7.0	8.25	1.2	8.00	1.1	9.26	1.3	7.70	1.1
Lys	5.5	3.46	0.6	3.24	0.5	3.40	0.6	3.03	0.6
Met + Cys (TSAA)	3.5	1.77	0.5	1.7	0.4	1.75	0.5	1.31	0.4
Phe + tyr	6.0	6.98	1.2	6.23	1.0	7.78	1.3	5.03	0.8
Thr	4.0	3.12	0.8	3.39	0.8	3.20	0.8	2.87	0.7
Try	1.0	ND	ND	ND	ND	ND	ND	ND	ND
Val	5.0	3.42	0.7	3.24	0.6	3.45	0.7	2.75	0.6
Total	36.0	30.01	5.8	28.59	5.1	32.36	6.1	25.39	4.9

PAAESP = Provisional amino acids egg scoring pattern; EAAC = Essential amino acids composition; AAS = Amino acids scores; ND = Not determined; Source = Belschant et al. [39]

Therefore, *B. eurycoma* under investigation satisfied the [32] requirements. Fermentation enhanced the concentration of arginine, threonine, valine, phenylamine, and leucine among the essential amino acids while alanine, serine and glutamic acid were better concentrated in the fermented sample among the non-essential amino acids. The roasted sample had the least concentration of the mentioned amino acids with the exception of serine. The reason for this may be attributed to loss of toxins such as trypsin inhibitors and haemagglutinin [36], or transamination and deamination reactions in the processed (roasted) seed of *B. eurycoma.*

The difference in amino acid composition (g/100 g cp) of raw and processed *B. eurycoma* is shown in Table 6. The amino acids in the fermented sample were better concentrated than in the samples processed by the other methods. Methionine and glycine showed an increase of 8.65% and 0.58% in the boiled sample, respectively. All amino acids in the roasted seed showed reduction, ranging from 6.89% in P-PER to 28.57% in methionine. There was no difference between the raw and fermented samples in glycine and cystine and between the raw and boiled sampled in methionine. The CV% ranged from 18.9 % in tyrosine to 93.1 % valine.

Table 7 displays the classification of the amino acid composition of *B. eurycoma.* The total amino acid (TAA) ranged from 62.52 to 74.94 g/100g cp. This is comparable with the result on some legume crops reported by some workers [24, 37]. The total essential amino acid (TEAA) with histidine ranged from 28.63 to 34.79 g/100g cp while, without histidine, it was in the range of 26.6– 30.37g/100 g cp. The total sulphur amino acid (TSAA) ranged from 1.31 to 1.77 g/100g cp, which is lower than 5.8 g/100g cp recommended for infants [32]. The essential aromatic amino acid (EArAA) obtained ranged from 3.03 to 4.13 g/100g cp, which is lower than the recommended range for ideal infant protein (6.8–11.8 g/100g cp) [32]. The highest value of EArAA was found in the fermented sample (4.13 g/100g cp). The range of total neutral, acidic and basic amino acid was 51.20–56.90%; 18.85–21.98%, and 13.80–14.20%, respectively, indicating that the totality of protein is probably acidic in nature. This is in accordance with the findings of [24]. The total non-essential amino acid (TNEAA) ranged from 33.91 to 40.15 g/100g cp while the %TNEAA was in the range of 53.60–54.70. The essential amino acid scores of the raw and processed samples of *B. eurycoma* seed based on the provisional amino acid scoring pattern [18] are depicted in Table 8. The most limiting amino acid in *B. eurycoma* was Met+Cys (TSAA) with the value ranging from 0.4 in the boiled and roasted samples to 0.5 in the raw and fermented samples. The present report thus contradicts with the findings of [38], who reported that Lys often acts as a limiting capacity. When comparing the essential amino acid in this report with the [18] recommended provisional pattern, *B. eurycoma* sample was only superior in Leu and Phe+Tyr while essential amino acid supplementation may be required in Met+Cys, Ile, Thr, Lys and Val. Try was not determined.

4. Conclusion

The proximate, mineral and amino acid compositions of raw and processed (*B. eurycoma*) seed flour were presented in this study. The study showed that *B. eurycoma* seeds have moderate fat and protein contents with nutritionally valuable minerals and useful amino acids comparable with the known protein-rich plant foods such as groundnut and soybean. It was found that the different processing methods employed in this study (boiling, fermentation and roasting) changed the nutrient content of the seeds. However, a more elaborate study to optimize the processing is required before an appropriate processing method can be recommended for the underutilized legume seeds.

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