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Influence of traditional processing methods on the nutritional composition of lack turtle bean (*Phaseolus vulgaris* L.) grown in Nigeria

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Abstract

Investigating the effect of different processing methods on nutritional composition of a lesser known crop (black turtle bean) was carried out with a view to providing adequate information towards effective utilization of this crop in various food applications in Africa and other parts of the world. The seeds of black turtle bean (*Phaseolus vulgaris* L.) were collected from Bokkos town of Plateau State, Nigeria and processed into raw dried, boiled, cooked, roasted, sprouted and fermented flours. Proximate, mineral and amino acid compositions were investigated using standard analytical techniques. The processing methods showed deviations in nutrients from the raw seeds. Crude protein was found to be enhanced by cooking (13.5%), roasting (4.64%) and sprouting (14.35%) methods while all the processing methods were found to reduce the contents of crude fat, fatty acids and metabolizable energy. Processing significantly ($p < 0.05$) affected the content of some minerals in *Phaseolus vulgaris* seeds. All the processing methods (boiling, cooking, roasting, sprouting and fermenting) increased magnesium, zinc and manganese contents by 44.16, 121.43 and 130.00%; 25.85, 100.00 and 150.00%; 35.54, 74.83 and 70.00%; 31.76, 107.14 and 110.00%; 31.28, 128.57 and 120.00%, respectively whereas sprouting decreased calcium and iron contents by 14.26 and 4.76%, respectively. Generally, raw and processed *Phaseolus vulgaris* seeds were found to be good sources of essential minerals and harmful lead was not at the detectable range of AAS. The amino acid profile revealed that all the processing methods increased the concentrations of total amino acid (TAA) while only cooking and roasting methods increased the concentrations of total essential amino acid (TEAA) with histidine by 1.46 and 1.95% compared with the raw value. The limiting amino acids (LAA) for both the raw and processed samples were either Met + Cys, Ile or Val. Sufficient proportions of the essential amino acids were retained after processing of the black turtle bean (*Phaseolus vulgaris*) to meet FAO/WHO dietary requirement.

Introduction

It is a well known fact that under–developed nations (*Nigeria inclusive*) all over the world do not produce enough food and of the right nutritive values to meet daily needs (Olaofe *et al*., 1993; Aremu *et al*., 2006a; Aremu *et al*., 2008). The dearth in food supply especially of protein is so enormous that the developing nations have to depend on cereals, grains, starch roots and tubers for energy and protein need (Akporhonor *et al*., 2006; Aremu *et al*., 2009). Legumes refer to the seeds of Leguminosae including peas, beans and pulses. Legumes are considered as "poor man's meat" due to their high protein content and low cost compared to meat and meat products (Balogun and Fetuga, 1986). Though legumes are important sources of dietary proteins for both human and animals, their usefulness have been hindered by the presence of some anti–nutritional factors known as toxins (Onyeike *et al*., 1995; Aremu *et al*., 2010). Nutritional quality is affected by these factors that interact with the intestinal tract such as phytate, tannins and oxalates which reduce protein digestibility and amino acid absorption (Nowacki, 1980; Davis, 1981). However, these substances need to be destroyed either by heat or other treatments otherwise concentration of toxins will exert adverse physiological effects when ingested by man and animals (Liener, 1994).

Black turtle bean (*Phaseolus vulgaris* L.), a herbaceous animal plant is thought to have originated from ancient Mesoamerica and the Andes and is now grown in many parts of the tropics including West Africa (Arkcoll and Clement, 1989). Black turtle beans are grown worldwide for its edibility and nutritional qualities. This crop is among lesser–known beans grown in Nigeria especially by the people of Bokkos in Plateau State. The leaf of black turtle bean

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is occasionally used as leaf vegetable while the plant can be taken in the form of stew and the straw as a folder. The industrial and nutritional potential of this crop is unknown to the host communities. Therefore, this work gives information on the effect of boiling, cooking, roasting, sprouting and fermenting on the chemical composition and nutritive value of black turtle (*Phaseolus vulgaris* L.) seeds which are grown in Plateau State, Nigeria.

Materials and Methods

Collection of the sample

For the purpose of this study, mature seeds of black turtle beans (*Phaseolus vulgaris*) were collected from a farmer in Bokkos town of Plateau State, Nigeria. The seeds were thoroughly cleaned and sorted to remove stones and bad ones. The processing methods employed were boiling, cooking, roasting, sprouting and fermenting while raw sample served as control.

Preparation of processed black turtle beans seed flours

Raw sample

Cold water was added on 500 g of black turtle bean seeds, left for 4 h and dehulled. The dehulled seeds were dried in the oven at 45°C.

Boiling

The dehulled raw black turtle beans (500 g) were boiled in distilled water at 100°C at the ratio of 1:10 wt/v for 45 min, after which they were drained and oven-dried at 50°C.

Cooking

The cooking was done in an aluminium pot using one part of the dehulled raw seeds (500 g) to 15 parts of distilled water on a Gallenkamp thermostat hot plate. The seeds were considered cooked when they became soft to touch when pressed between the thumb and fingers. At the end of the cooking time, the boiling water was drained and seeds were sun-dried.

Roasting

Raw seeds (500 g) were manually dehulled, roasted in fine sand and stirred using the Gallenkamp thermostat hot plate 85°C until a characteristic brownish coloured seed was obtained after 1 h 20 min, which indicated complete roasting. Then, the seeds were cleaned and cooled.

Sprouting

Black turtle bean seeds were germinated using sawdust in a locally woven reed basket. The seeds were arranged in layers of sawdust, wetted daily and observed for sprouting. Seeds with sprouts about 1 cm long (3 – 4 days) were picked, washed, dehulled, sliced and dried at 40°C.

Fermenting

The dehulled raw seeds (500 g) were wrapped in blanched banana leaves and allowed to ferment for 4 days. Fermented seeds were picked, washed, sliced and dried at 40°C. After all processing treatments were completed; all the raw and processed seed samples were ground into fine flour with a small sample mill (DIETZ, 7311 Dettingentech, West Germany). They were kept in airtight container and put in a deep freezer (-6°C) prior to chemical analyses.

Proximate analyses

The moisture, ash, crude fat, crude protein (N × 6.25), crude fibre and carbohydrate (by difference) were determined in accordance with AOAC (1980) methods. All proximate analyses of the legume flours were carried out in triplicate and reported in percent. All chemicals were of Analar grade.

Mineral analysis

Potassium and sodium were determined using a flame photometer (Corning, UK Model 405) and KCl and NaCl were used to prepare the standards. Phosphorus was determined by vanadomolybdate colourimetric method (James, 1996). All other metals were determined by atomic absorption spectrophotometer (Perkin-Elmer Model 403, Norwalk CT). All determinations were done in triplicate and the minerals were reported in mg/100 g.

Amino acid analysis

The amino acids were analysed by ion exchange chromatography (IEC) (FAO/WHO, 1991) 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^{-1} at 60°C with reproducibility consistent within ±3%. 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.

Determination of quality parameters

Determination of isoelectric point (pI): The predicted isoelectric point was evaluated as follows (Olaofe and Akintayo, 2000):

$$p\,\mathrm{Im} = \sum_{i=1}^{n=1} pIiXi$$

Where pIm is the isoelectric point of the mixture of amino acids, pI_i is the isoelectric point of the i^{th} amino acids in the mixture and X_i is the mass or mole fraction of the amino acids in the mixture.

Determination of amino acid scores

Amino acid scores were determined based on whole hen's egg (Paul *et al.*, 1976). In this method, essential amino acids were scored, Met + Cys and Phe + Tyr were taken as a unit while amino acid score (AAS) was calculated using the following formula (FAO/WHO, 1973):

$$amino\ acid\ score\,(AAS) = \frac{amount\ of\ amino\ acid\ per\ test\ protein\,(mg\ g^{-1})}{amount\ of\ amino\ acid\ per\ protein\ in\ reference\ pattern\,(mg\ g^{-1})}$$

Determination of the predicted protein efficiency ratio

The predicted protein efficiency ratio (P-PER) of differently processed samples was calculated from their amino acid composition based on the following equation (Alsmayer *et al.*, 1974):

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

Calculations and statistical analyses

Sodium/potassium (Na/K) and calcium/ phosphorus (Ca/P) ratios were calculated for the samples (Nieman *et al.*, 1992). The fatty acid values were obtained by multiplying crude fat value of each sample with a factor of 0.8 (i.e. crude fat × 0.8 = corresponding to fatty acids value) (Paul and Southgate, 1985). The energy values were calculated by adding up the carbohydrate × 17 kJ, crude protein × 17 kJ and crude fat × 37 kJ for each of the samples (Kilgour, 1987). Standard deviations were calculated using MS Excel Spread sheet from the three determinations done on each sample for the proximate composition and it was used as the measure of dispersion.

Results and Discussion

The 4.2% moisture content of black turtle bean seeds obtained for the raw sample (Table 1) is higher than values obtained for *Proposis africana* (3.32%) (Vidivel and Janardhanen, 2001a), *P. Coccineus* (3.7%) (Aremu *et al.*, 2010), *Canavalia ensiformis* 3.8% (Vidivel and Janardhanen, 2001) and pinto beans 2.30% (Audu and Aremu, 2011). However, it is within the expected values of most legumes. It is lower than values reported for the yara-1 variety of kidney bean (6.50%) (Ejigui *et al.*, 2005) and that of cowpea (9.20%) (Giami, 1993) but compared favourably to that of cream coat cowpea (4.0%) (Olaofe and Sanni, 1988; Aremu *et al.*, 2006a). The low moisture value of the black turtle bean remains an asset in storage and preservation of the nutrients. Onyeike *et al.* (1995) observed that higher moisture content could lead to food spoilage through increasing microbial action. The ash content of 4.9% (Table 1) observed is moderate but higher than those of the wild jack bean (3.0%) (Vidivel and Janardhanen, 2001), melon seeds (3.3%) (Omafuvbe *et al.*, 2004), *Vigna ublobata* (3.2%) and *Vigna radiate* (3.4%) (Khalil and Khan, 1995), castor seeds (*Ricinus communis*) (3.2%) (Onyeike and Acheru, 2002). Since the sample contained fairly high ash content, it may indicate that the legume could provide essential valuable and useful minerals needed for good body development since soya bean which occupies a unique position among leguminous crops has an average ash content of 4.2% (Temple *et al.*, 1991). The crude fat content of the seed flour (15.2%) of the raw sample compared with those of other legumes such as red kidney bean (14.4%) (Olaofe *et al.*, 2010), *Prosopis africana* (12.93%) (Barminas *et al.*, 1998). The values are higher than those observed for cowpea (3.1%), scarlet runner bean (7.5%) Kersting's groundnut (5.9%) and bambara groundnut (6.7%) (Aremu *et al.*, 2006a) and slightly lower than that reported for soya bean (19.4%) by Temple *et al.* (1991). This indicates that the black turtle bean is a better source of oil than cowpea, *P. Africana*, scarlet runner bean, kersting's groundnut and bambara groundnut. Hence black turtle bean can be considered as a potential vegetable oil seed for the future for domestic and industrial purposes.

The crude protein value for the sample is 23.70%. This value suggests that black turtle bean can contribute to the daily protein need of 23.6 g for adults, as recommended by the National Research Council (1974). This result compared well with selected legumes such as African locust bean (*Parkia bigbosa*) (31.0%) (Omafuvbe *et al.*, 2004), wild Jack bean (28.9%) (Vidivel and Janardhanen, 2001), red kidney bean (28.5%) (Olaofe *et al.*, 2010). The crude fibre value of 3.3 for the raw to 6.4% in fermented compared favourably with ranges reported for cowpea (2.6%) (Aletor and Aladetimi, 1989), red kidney bean (2.65%) (Olaofe *et al.*, 2010), cowpea (2.10%) (Gami, 1993), cream coat bambara groundnut (2.1%) (Aremu *et al.*, 2006a). The value of raw is lower than those of soya bean (4.28%) (Temple *et al.*, 1991), lima bean (5.4%), pigeon pea (4.8%) and Jack bean (9.5%) (Apata and Ologhobo, 1994). This

Table 1. Proximate composition (%) of raw and processed black turtle bean seed flours

Parameter	Raw I	Boiled II	Cooked III	Roasted IV	Sprouted V	Fermented VI	Mean	SD	CV%
Moisture	4.2 (0.30)	5.2 (0.50)	3.6 (0.25)	8.3 (0.20)	5.8 (0.25)	8.5 (0.02)	5.9	1.88	31.86
Ash	4.9 (0.10)	3.2 (0.20)	3.1 (0.30)	3.9 (0.01)	3.4 (0.03)	3.2 (0.01)	5.9	3.09	52.37
Crude fat	15.2 (0.05)	14.7 (0.15)	13.7 (0.00)	10.2 (0.06)	10.0 (0.10)	10.5 (0.15)	10.1	5.04	49.90
Crude protein	23.7 (0.15)	22.3 (0.30)	26.9 (0.04)	24.8 (0.50)	27.1 (0.10)	22.0 (0.55)	24.5	2.01	8.20
Crude fibre	3.3 (0.01)	3.5 (0.04)	5.5 (0.10)	3.7 (0.10)	4.1 (0.00)	6.4 (0.01)	4.40	1.14	25.91
Carbohydrate[y]	48.8 (0.25)	51.1 (0.05)	47.3 (0.20)	49.1 (0.15)	49.6 (0.25)	49.6 (0.10)	49.3	1.13	2.29
Energy[w]	1793.7	1791.3	1767.1	1635.3	1429.6	1334.7	1625.3	182.06	11.20
Fatty acid[z]	12.1	11.8	10.9	8.2	2.7	2.5	8.0	4.04	50.50

[x]Number in parentheses are standard deviations of triplicate determinations;
[y]Carbohydrate percent calculated as the (100-total of other components)
[w]Calculated metabolizable energy (kJ/100 g) (protein x 17 + fat x 37 + carbohydrate x 17)
[z]Calculated fatty acids (0.8 x crude fat)
SD = Standard deviation
CV% = Coefficient of variation

Table 2. Differences in proximate composition between raw and processed black turtle bean seed flours

Parameter	I-II	I-III	I-IV	I-V	I-VI	Mean	SD	CV%
Moisture	-1.0(-23.81%)	0.6(14.29%)	-4.1(-86.67%)	-1.6(-38.10%)	-4.3-102.38%)	2.3	1.57	68.26
Ash	1.7(34.81%)	1.8(36.74%)	1.0(20.41%)	-5.0(-102.04%)	-5.5(-112.25%)	3.0	1.86	62.00
Crude fat	0.5(3.29%)	1.5(9.87%)	5.0(32.89%)	11.8(77.63%)	12.0(78.95%)	6.2	4.92	79.35
Crude protein	1.4(5.91%)	-3.2(-13.5%)	-1.1(-4.64%)	-3.4(-14.35%)	1.7(7.17%)	2.2	0.95	43.18
Crude fibre	-0.2(-6.06%)	-0.2(-6.06%)	-0.4(-12.12%)	-0.8(-24.24%)	-3.1(-93.93%)	0.9	1.10	122.22
Carbohydrate	-2.3(-4.71%)	1.5(3.07%)	-0.3(-0.01%)	-0.8(-1.64%)	-0.8(-1.64%)	1.1	0.70	63.64
Energy	2.4(0.13%)	26.6(1.48%)	158.4(0.01%)	364.1(20.30%)	459(25.59%)	202.1	181.57	89.84
Fatty acid	0.3(2.48%)	1.2(9.92%)	3.9(32.23%)	9.4(77.69%)	9.6(79.34%)	4.9	3.95	80.61

I = Raw; **II** = Boiled; **III** = Cooked; **IV** = Roasted; **V** = Sprouted; **VI** = Fermented; **SD** = Standard deviation; **CV%** = Coefficient of variation

Table 3. Mineral composition (g/100 g DM) of raw and processed black turtle bean seed flours

Mineral	I	II	III	IV	V	VI	Mean	SD	CV%
Mg	600.1	865.0	755.1	813.3	790.6	787.7	768.6	82.38	10.72
Ca	49.1	65.5	42.1	58.2	66.2	60.1	56.9	8.68	15.25
Ni	4.0	4.2	3.5	3.8	4.5	4.7	4.1	0.41	10.00
Zn	1.4	3.1	2.8	2.5	2.9	3.2	2.7	0.60	22.22
Mn	0.9	2.2	2.4	1.6	2.0	2.1	1.9	0.50	26.32
Cu	0.3	1.0	0.7	0.7	0.7	0.9	0.70	0.22	31.43
Fe	10.5	12.5	10.0	13.5	15.2	12.9	12.4	1.76	14.19
Cr	2.8	2.0	3.0	1.9	1.8	2.1	2.3	0.46	20.00
Co	ND	ND	ND	ND	ND	ND	-	-	-
Pb	ND	ND	ND	ND	ND	ND	-	-	-
Na	24.6	33.4	28.5	29.9	26.6	17.6	26.8	4.86	18.13
K	14.1	13.0	15.9	14.2	10.1	19.6	14.5	2.88	19.86
P	2.4	3.0	3.9	3.5	2.4	4.5	3.3	0.77	23.33
Na/K	1.8	1.8	1.8	2.1	2.6	0.9	1.8	0.51	28.33
Ca/P	20.5	22.2	11.9	16.9	28.2	13.4	18.9	5.53	29.26

ND = not detected; **Ca/P** = calcium to phosphorus ratio; **Na/K** = sodium to potassium ratio; **I** = Raw; **II** = Boiled; **III** = Cooked; **IV** = Roasted; **V** = sprouted; **VI** = Fermented;

SD = standard deviation; **CV** = coefficient of variation; – = not determined

suggests that the sample would provide moderate dietary fibre in the diet. Fibre helps to maintain the health of the gastrointestinal tract, but in excess may bind trace elements, leading to deficiencies of iron and zinc (Siddhuraju *et al*., 1996). The carbohydrate content 48.8% in raw to 51.1 in boiled sample was comparable with those of *P. africana* (41.61%) (Barminas *et al*., 1998) varieties of Sesbania seeds (44.6-47.4%) (Hossain and Becker, 2001), brown wrinkled pigeon pea (40.4%) and Olaludi (brown) variety of cowpea (40.5%) (Ene-Obong and Carnovale, 1992). The value is however higher than those of soya bean (26.3%) as reported by Temple *et al.* (1991), cranberry bean (31.5%), red specks coat and white coat scarlet runner bean, 31.4% and 30.1%, respectively (Aremu *et al*., 2006b) but lower to those reported for Yara-1 variety of kidney bean (66.9%) (Apata and Ologhobo, 1994), cream coat bambara groundnut (73.9%), kerstings groundnut (77.3%), moderate brown coat cowpea (82.9%) and small white coat cowpea (77.2%) (Aremu *et al*., 2006a). The carbohydrate content suggests that the seed could be a good supplement to scarce cereal grains as source of energy and feed formulations. The energy value was 1429.6 kg/100 g in sprouted to 1793.7 kg/100 g in raw samples. The 1793.7 kg/100 g in the raw compared well with selected legumes such as *Acacia nilotica* (1532.5 kg/100 g) (Siddhuraju *et al*., 1996), *Canavalia ensiformis* (1469-1574 kg/100 g) (Vidivel and Janardhanen, 2001), bambara groundnut (1691.3 kg/100 g), kersting's groundnut (1692.9 kg/100 g) and cranberry beans (1651.7 kg/100 g) (Aremu *et al*.,

Table 4. Differences in mineral composition (g/100 g DM) between raw and processed black turtle bean seed flours

Mineral	I-II	I-III	I-IV	I-V	I-VI	Mean	SD	CV%
Mg	-265.0(-44.16%)	-155.1(-25.85)	-213.3(-35.54%)	-190.6(-31.76%)	-189.7(-31.28%)	202.2	36.73	18.17
Ca	-16.4(-33.40%)	7.0(14.26%)	-9.4(-19.15%)	-17.1(-34.83%)	-11.0(-22.40%)	12.2	3.95	32.38
Ni	-0.20.5(-5.00%)	0.5(12.50%)	0.2(5.00%)	-0.5(-12.50%)	-0.7(-17.50%)	0.4	0.19	47.50
Zn	-1.7(-121.43%)	-1.4(-100.00%)	-1.1(-74.83%)	-1.5(-107.14%)	-1.8(-128.57%)	1.5	0.24	16.00
Mn	-1.3(-130.00%)	-1.5(-150.00%)	-0.7(-70.00%)	-1.1(-110.00%)	-1.2(-120.00%)	1.2	0.61	42.3
Cu	-0.7(-233.33%)	-0.4-(-133.33%)	0.4(133.33%)	-0.4(-133.33%)	-0.6(-200.00%)	0.5	0.13	26.00
Fe	-2.0(-19.05%)	0.5(4.76%)	-3.0(-28.57%)	-4.7(-44.76%)	-2.4(-22.86%)	2.5	1.37	54.80
Cr	0.8(28.5%)	-0.2(-7.14%)	0.9(32.14%)	1.0(35.71%)	0.7(25.00%)	0.7	0.28	40.00
Co	ND	ND	ND	ND	ND	-	-	-
Pb	ND	ND	ND	ND	ND	-	-	-
Na	-8.8(-35.77%)	-3.9(-15.85%)	-5.3(-21.55%)	-2.0(-8.13%)	7.0(28.46%)	5.4	2.36	43.73
K	1.1(7.80%)	-1.8(-12.77%)	-0.1(-0.71%)	4.0(28.37%)	-5.5(-39.01%)	2.5	1.97	78.80
P	-0.6(-25.00%)	-1.5(--62.50%)	-1.1(-45.83%)	0.0(0.00%)	-2.1(-87.50%)	1.1	0.53	48.18
Na/K	0.0(0.00%)	0.0(0.00%)	-0.3(-16.67%)	-0.8(-44.44%)	0.9(50.00%)	0.4	0.29	72.50
Ca/P	-1.7(-8.29%)	8.6(41.95%)	3.6(17.56%)	-7.7(-37.56%)	7.1(34.63%)	5.7	3.03	53.16

-= not determined; **Ca/P** = Calcium to phosphorus ratio; **Na/K** = Sodium to potassium ratio; **I** = Raw; **II** = Boiled; **III** = Cooked; **IV** = Roasted; **V** = Sprouted; **VI** = Fermented; **SD** = Standard deviation; **CV** = Coefficient of variation; **ND** = not detected

Table 5. Amino acid composition (g/100 g crude protein DM) of raw and processed black turtle bean seed flours

Amino Acid	I	II	III	IV	V	VI	Mean	SD	CV%
Lysine (Lys)[y]	6.5	5.9	7.7	7.2	6.9	6.8	6.8	0.56	8.24
Histidine (His)[y]	3.2	3.3	3.2	3.4	3.4	3.1	3.3	0.12	3.64
Arginine (Arg)[y]	6.5	6.2	6.6	6.2	6.6	6.9	6.3	0.26	4.13
Aspartic acid (Asp)	7.9	8.0	8.0	8.7	9.7	8.6	8.5	0.63	7.41
Threonine (Thr)[y]	2.9	3.4	3.4	3.6	3.1	2.9	3.2	0.27	8.44
Serine (Ser)	3.6	3.4	3.4	2.7	2.9	3.1	3.2	0.31	9.97
Glutamic acid(Glu)	11.2	13.5	10.2	11.2	11.6	11.2	11.5	1.00	8.70
Proline (Pro)	3.0	3.2	3.7	3.5	2.9	2.9	3.2	0.31	9.97
Glycine (Gly)	4.0	3.3	3.9	4.2	3.5	3.6	3.8	0.31	8.22
Alanine (Ala)	3.2	4.5	4.2	4.0	4.2	4.1	4.0	0.40	10.00
Cystine (Cys)	0.8	1.2	1.5	1.2	0.8	1.3	1.1	0.26	23.64
Valine (Val)[y]	4.7	4.1	3.6	3.9	4.2	4.2	4.1	0.33	8.05
Methionine (Met)[y]	1.3	1.2	1.3	1.6	1.5	1.3	1.4	0.14	10.00
Isoleucine (Ile)[y]	4.1	3.6	3.6	3.1	3.6	3.2	3.5	0.33	9.43
Leucine (Leu)[y]	7.7	6.9	7.6	8.2	6.8	7.5	7.5	0.48	6.40
Tyrosine (Tyr)	3.5	3.1	3.5	3.2	3.1	3.4	3.3	0.17	5.15
Phenylalanine (Phe)[y]	4.2	4.9	4.7	4.7	3.9	5.3	4.6	0.46	10.00
Isoelectric point (pI)	4.7	4.7	4.9	4.8	4.7	4.7	4.8	0.96	18.96
P-PER	2.7	2.4	2.6	2.9	2.3	2.6	2.6	0.20	7.69
Leu/Ile	1.9	2.0	2.1	2.7	1.9	2.4	2.2	0.26	11.82

[y]Essential amino acid; **I** = Raw; **II** = Boiled; **III** = Cooked; **IV** = Roasted; **V** = Sprouted; **VI** = Fermented; **SD** = Standard deviation; **CV** = Coefficient of variation; **P-PER** = Calculated predicted protein efficient ratio; **Leu/Ile** = Leucine to isoleucine ratio.

2006b). This observation reflects that, like other food legumes, black turtle bean seed could be a rich source of energy. The contribution of energy from the seed is important since sufficient supply of energy in the diet is required for protein to be fully utilized (Worgan, 1973).

Table 2 shows the differences between the raw and the processed samples (boiling, cooking, roasting, sprouting and fermenting). Processing affected the proximate composition of the black turtle seed flour for instance, it was observed that there was difference in moisture content up to 14.29% in cooking to 102.38% in fermenting by either an increase or decrease from that of the raw seed. The water soluble ash ranged from 20.41% in roasting to 112.25% in fermenting. All the processing methods in this work were found to reduce crude fat, energy and fatty acid while crude fibre was increased. The increase in crude fibre of the processed seeds may be due to the utilization of the sugar in the seed for metabolic activity resulting in higher percentage of the fibre. Crude protein was increased by cooking, roasting and sprouting while boiling and fermenting reduced it. This is in contrast to the observation reported for mung bean (Del Rosario and Flores, 1981), cereals (Wang and Fields, 1978), soya bean (Kylen and McCready, 1975) and sorghum (Asiedu *et al.*, 1993) that only sprouting increases crude protein content. The increased crude protein in the sprouting sample can be attributed to protein synthesis during germination while the reduction in boiled can be as a result of leaching of soluble components and metabolic activity in fermenting (Kylen and McCready, 1975). The CV% varied with a range of 43.18% in crude protein to 122.22% in crude fibre (Table 2).

The mineral profile of black turtle bean is presented in Table 3. Of all the minerals determined, magnesium is the most abundant having a value range of 600.1 mg/100 g in the raw to 865.0 mg/100 g in the boiled sample. This observation is contrary to observation reported for most legumes that potassium is the most abundant (Olaofe and Sanni, 1988). It is followed by calcium (49.1 mg/100 g), sodium (24.6 mg/100 g) and potassium (14.1 mg/100 g). The seeds of black turtle bean were also rich sources of the following nutritional valuable minerals, Fe (10.5 mg/100 g), Ni (4.0 mg/100 g), Cr (2.8 mg/100 g), P (2.4 mg/100 g) and Zn (1.4 mg/100 g). Magnesium is required for bone formation which maintains the electrical potential in nerves (Shils and Young, 1998). The calcium and phosphorus levels are reasonably distributed in the sample. Phosphorus is always found with calcium in the body both contributing to the blood formation and supportive structure of the body

Table 6. Differences in amino acid composition (g/100 g crude protein DM) of raw and processed black turtle bean seed flours

Amino Acid	I-II	I-III	I-IV	I-V	I-VI	Mean	SD	CV%
Lys[y]	0.6(9.23%)	-1.2(-18.46%)	-0.7(-10.77%)	-0.4(-6.15%)	-0.3(-4.62%)	0.6	0.32	53.33
His[y]	-0.1(-3.13%)	0.0(0.00%)	-0.2(-6.25%)	-0.2(-6.25%)	0.1(3.13%)	0.1	0.06	60.00
Arg[y]	0.3(4.62%)	-0.1(-1.54%)	0.3(4.62%)	-0.1(-1.54%)	0.6(9.23%)	0.3	0.18	60.00
Asp	-0.1(-1.27%)	-0.1(-1.27%)	-0.8(-10.13%)	-1.8(-22.78%)	-0.7(-8.86%)	0.7	0.62	88.57
Thr[y]	-0.5(-17.24%)	-0.5(-17.24%)	-0.7(-24.14%)	-0.2(-6.90%)	0.0(0.00%)	0.4	0.17	42.50
Ser	0.2(5.55%)	0.2(5.55%)	0.9(25.00%)	0.7(19.44%)	0.5(13.88%)	0.5	0.28	56.00
Glu	-2.3(-20.53%)	1.0(8.93%)	0.0(0.00%)	-0.4(-3.57%)	0.0(0.00%)	0.7	0.74	105.71
Pro	-0.2(-6.67%)	-0.7(-23.33%)	-0.5(16.67%)	0.1(3.33%)	0.1(3.33%)	0.3	0.24	80.00
Gly	0.7(17.5%)	0.1(2.5%)	-0.2-12.50%)	0.5(12.50%)	0.4(10.00%)	0.4	0.21	52.50
Ala	-1.3(-40.63%)	-0.1(-3.13%)	-0.8(-25.00%)	-0.1(-3.13%)	-0.9(-28.13%)	0.6	0.47	78.33
Cys	-0.4(-50.00%)	-0.7(-87.50%)	-0.4(-50.00%)	0.0(0.00%)	-0.5(-62.13%)	0.4	0.14	35.00
Val[y]	0.6(12.77%)	1.1(23.40%)	0.8(17.02%)	0.5(10.64%)	0.5(10.50%)	0.7	0.23	32.86
Met[y]	0.1(7.69%)	0.0(0.00%)	-0.3(-23.08%)	-0.2(-15.39%)	0.0(0.00%)	0.1	0.1	100.00
Ile[y]	0.5(12.20%)	0.5(12.20%)	1.0(24.39%)	0.5(12.20%)	0.9(21.95%)	0.7	0.22	31.43
Leu[y]	0.8(10.39%)	0.1(1.30%)	-0.5(-6.49%)	0.8(10.40%)	0.2(2.60%)	0.5	0.29	58.00
Tyr	0.4(11.43%)	0.0(0.00%)	0.3(8.57%)	0.4(11.43%)	0.1(2.86%)	0.2	0.14	70.00
Phe[y]	-0.7(-16.67%)	-0.5(11.90%)	-0.5(11.90%)	0.3(7.14%)	-1.1(-26.19%)	0.6	0.27	45.00
pI	0.0(0.00%)	-0.2(-4.26%)	-0.1(-2.13%)	0.0(0.00%)	0.0(0.00%)	0.1	0.04	40.00
P-PER	0.3(11.11%)	0.1(3.70%)	-0.2(-7.41%)	0.4(14.82%)	0.1(3.70%)	0.2	0.12	60.00
Leu/Ile	-0.1(-5.88%)	-0.2(-11.77%)	-0.8(-47.06%)	0.0(0.00%)	-0.5(-29.41%)	0.3	0.26	86.67

[y]Essential amino acid; **I** = Raw; **II** = Boiled; **III** = Cooked; **IV** = Roasted; **V** = Sprouted; **VI** = Fermented; **SD** = Standard deviation; **CV** = Coefficient of variation; **P-PER** = Calculated predicted protein efficient ratio; **Leu/Ile** = Leucine to isoleucine ratio.

Table 7. Concentrations of essential, non-essential, acidic, neutral, sulphur, aromatic, etc. (g/100 g crude protein DM) processed black turtle bean flour

Amino Acid Description	I	II	III	IV	V	VI
Total Amino Acid (TAA)	78.3	79.7	80.1	80.6	78.7	78.4
Total Non-Essential Amino Acid (TNEAA)	37.2	40.2	38.4	38.7	38.7	38.2
% TNEAA	47.5	50.4	47.9	48.0	49.2	48.7
Total Essential Amino Acid (TEAA)						
With Histidine	41.1	39.5	41.7	41.9	40.0	40.2
Without Histidine	37.9	36.2	38.5	38.5	36.6	37.1
% TEAA						
With Histidine	52.5	49.6	52.1	52.0	50.8	51.3
Without Histidine	48.4	45.4	48.1	47.8	46.5	47.3
Essential Alphatic Amino Acid (EAAA)	19.4	18.0	18.2	18.8	17.7	17.8
Essential Aromatic Amino Acid (EArAA)	4.2	4.9	4.7	4.7	3.9	5.3
Total Neutral Amino Acid (TNAA)	43.0	42.8	44.1	43.9	40.5	42.4
% TNAA	54.9	53.7	55.1	54.5	51.5	54.1
Total Acidic Amino Acid (TAAA)	19.1	21.5	18.2	19.9	21.3	19.8
% TAAA	24.4	26.9	22.7	24.7	27.1	25.3
Total Basic Amino Acid (TBAA)	16.2	15.4	17.5	16.8	16.9	15.8
% TBAA	20.7	19.3	21.8	20.8	21.5	20.2
Total Sulphur Amino Acid (TSAA)	2.1	2.4	2.8	2.8	2.3	2.6
% Cystine in TSAA	38.1	50.0	53.6	42.9	34.8	50.0

I = Raw; **II** = Boiled; **III** = Cooked; **IV** = Roasted; **V** = Sprouted; **VI** = Fermented.

(Ogunlade *et al*., 2005). Low Ca:P ratio facilitates decalcination of calcium in the bone leading to low calcium level in the bones while Ca/P ratio above two helps to increase the absorption of calcium in the small intestine (Nieman *et al*., 1992). The values of Ca/P ratio in the present study are far greater than two ranged from 11.9 in cooked sample to 28.2 in the sprouted sample therefore raw and processed samples of black turtle bean will participate well in these functions. The ratio of sodium to potassium in the body is of great concerned for the prevention of high blood pressure. Na/K ratio less than one is recommended (Aremu *et al*., 2006a). The Na/K ratio values ranged from 0.9 in fermented to 2.6 in sprouted samples (Table 3). This indicates that regular consumption of black turtle bean may not prevent high blood pressure. The CV% ranged from 10.00 in nickel to 31.43 in copper. Harmful metals such as cobalt and lead were not at detectable range of AAS. Processing significantly affects most of the mineral content of the seed flour (Table 4). All the processing methods (boiling, cooking, roasting, sprouting and fermenting) increased mineral contents such as magnesium (25.85% in cooked to 44.16% in boiled samples), zinc (74.83% in roasted to 128.57% in fermented), manganese (70.0% in roasted to 150% in cooked sample). Copper followed the same trend of observation in magnesium: Boiling, roasting, spouting and fermenting increased calcium content by 33.40, 19.45, 34.83 and 22.40%, respectively while there was a reduction by cooking which could be as a result of the effect of heat changing the insoluble chemical species of the some trace elements into soluble ones, which were extracted in the cooking

Table 8. Amino acid Score of raw and processed black turtle bean

EAA	PAAESP[a] (g/100 g protein)	Raw		Boiled		Cooked		Roasted		Sprouted		Fermented	
		EAAC	AAS	EAAC	AAS	EAAC	AAS	EAAC	AAS	EAAC	AAS	EAAC	AAS
Ile	4.0	4.1	1.0	3.6	0.9	3.6	0.9	3.1	0.8	3.6	0.9	3.2	0.8
Leu	7.0	7.1	1.1	6.9	1.0	7.6	1.1	8.2	1.2	6.8	1.0	7.5	1.1
Lys	5.5	6.5	1.2	5.9	1.1	7.7	1.4	7.2	1.3	6.9	1.3	6.8	1.2
Met + Cys (TSAA)	3.5	2.1	0.6	2.4	0.7	2.8	0.8	2.8	0.8	2.4	0.7	2.6	0.7
Phe + Tyr	6.0	7.7	1.3	8.3	1.4	8.1	1.4	8.3	1.4	7.0	1.2	8.2	1.4
Thr	4.0	2.9	0.7	3.4	0.9	3.4	0.9	3.6	0.9	3.1	0.8	2.9	0.7
Try	1.0	-	-	-	-	-	-	-	-	-	-	-	-
Val	5.0	4.7	0.9	4.1	0.8	3.6	0.7	3.9	0.8	4.2	0.8	4.2	0.8
Total	**36.0**	**35.1**	**5.9**	**34.6**	**6.7**	**36.8**	**7.2**	**37.1**	**7.1**	**34.0**	**6.7**	**35.4**	**6.8**

EAA = Essential amino acid; **PAAESP** = Provisional amino acid (egg) scoring pattern; **EAAC** = Essential amino acid composition (see Table 5); **AAS** = Amino acid scores; -= Not determined; [a]**Source** = Belschant *et al.* (1975).

water (Duhan *et al.*, 2000).

Table 5 presents the amino acid profile of the black turtle bean seed flour, the present result indicates that glutamic acid (11.2 mg/100 g crude protein) and aspartic acid (7.9 g/100 g crude protein, cp) were the major amino acids in the black turtle bean. The values are very low compared to values reported for red kidney beans that is glutamic acid (13.4 g/100 g cp) and aspartic acid (9.67 g/100 g cp) by Olaofe *et al*. (2010). However, this observation was in a close agreement with the observation of Oshodi *et al*. (1993); Olaofe *et al.* (1994); Adeyeye (2006); Aremu *et al.* (2011) that glutamic acid is the most abundant amino acid followed by aspartic acid. Leucine (7.7 g/100 g cp), lysine and argine (6.5 g/100 g cp) were the most concentrated essential amino acids. Methionine content (1.3 g/100 g cp) of the black turtle bean seed is lower than that of soya bean (9.0 g/100 g cp) (Temple *et al.*, 1991). Trytophan was not determined. The predicted protein efficiency ratio (P-PER) is one of the quality parameters used for protein evaluation (FAO/WHO/UNU, 1985). The P-PER values in this report (2.3-2.7) are higher than that of cowpea (1.21) (Aremu *et al.*, 2006b) and millet ogi (1.62) (Oyarekua and Eleyinmi, 2004). Thus the raw and processed seeds of black turtle bean under investigation meet the FAO standard (FAO/WHO/UNU, 1985). The calculated isoelectric point (pI) ranged from 4.7-4.9. This is useful in predicting the pI for protein in order to enhance a quick precipitation of protein isolate from biological samples (Aremu *et al.*, 2006a). The Leu/Ile ratios in all the processed seeds were low (1.9-2.7). Despite processing and its effect, the coefficient of variation (CV%) levels were very close with a stronger spot at 23.64 in cystine and others ranged from 3.64 to 18.96.

Differences in the mean amino acid composition of raw and processed black turtle bean seeds are shown in Table 6. Cooking, roasting, sprouting and fermenting increased lysine while boiling reduced it by 9.23%. Val and Ile were significantly affected by all the processing methods by a total reduction of 12.77, 23.40, 17.02, 10.64 and 10.50% (boiling, cooking, roasting, sprouting and fermenting) for Val while Ile had a reduction 12.20% for boiling, cooking and sprouting; 24.29% (roasting) and 21.950% (fermenting). Transamination and deamination reactions might be responsible for the slight changes in the amino acid profiles of raw and processed black turtle seed flour. The calculated Isoelectric point (pI) recorded increase only in cooked and roasted samples while increase in P-PER value up to 7.41% and decrease (14.82%) were observed. The CV% were highly varied with the highest variability recorded in Glu (105.71).

Evaluation report on amino acid based on classification, of different processed black turtle seeds is shown in Table 7. Total amino acid (TAA), total essential amino acid (TEAA) with His and total sulphur amino acids (TSAA) of raw seeds were 78.3, 41.1 and 2.1 g/100 g cp, respectively. All the processing methods (boiling, cooking, roasting, sprouting and fermenting) enhanced TAA and TSAA while TEAA with His was also enhanced by cooking, roasting and fermenting but reduced by boiling and sprouting. The TSAA of all the processed seeds are lower than the 5.8 g/100 g cp recommended for infants (FAO/WHO/UNU, 1985). The essential aromatic amino acid (EArAA) varied between 3.9 g/100 g cp in sprouted seeds to 5.3 g/100 g cp in fermented seeds. These values are lower than values reported for cranberry bean (9.9-12.2 g/100 g cp) (Aremu *et al.*, 2010). The values in this study do not meet up the range suggested for infant protein (6.8-11.8 g/100 g cp). Table 7 also shows the TAAA (19.1-21.5) and TBAA (15.4-17.5). The values showed that TAAA are greater than TBAA indicating that the protein is probably acidic in nature (Aremu *et al*., 2006a). The TEAA (%) ranged from 49.6% in boiled seed to 52.1% in cooked seed. These values are comparable with that of egg (50%) (FAO/WHO, 1991) and beach pea protein isolate (44.4%) (Chavan *et al*., 2001).

Table 8 presents the EAA scores of the raw and processed samples based on the provisional amino acid sccoring patern (FAO/WHO, 1991). With exception of Ile, Leu, Lys and Phe + Tyr in all the processed samples (boiling, cooking, roasting, sprouting and fermenting), the amino acid score in the processing method was that early in the cooking/boiling process for example, there is loss of toxic activity particularly of the proteinous toxins, trypsin inhibitors and haemagglutinnins (Kingsley, 1995). Although as heating proceeds, protein quality increases to a maximum, with continued heating it is reduced, this reduction is likely to be related to increasing Maillard browning causing lysine to be rendered unavailable (Chavan *et al.*, 2001). By implication, dietary formular based on the raw processed black turtle bean seed in this report will require some essential amino acid supplementations such as Met + Cys (TSAA), Thr and Val for the raw seed; Ile, Met + Cys (TSAA), Thr and Val for all the processing methods. It has been reported that the essential amino acid scores most often acting in limiting capacity are Met + Cys, Lys and Try (Salunkhe and Kadam, 1989; Aremu *et al.*, 2007; Aremu and Ekunode, 2008). However, in this report TSAA (Met + Cys) was the first limiting amino acid for the raw and all the processed samples except cooking which has Val as limiting amino acid.

Conclusion

This work has presented influence of domestic processing methods (boiling, cooking, roasting, sprouting and fermenting) on the chemical composition of black turtle bean (*Phaseolus vulgaris* L.) seeds grown in Plateau State, Nigeria. The study showed that black turtle bean seeds have high protein and energy contents with nutritionally valuable minerals comparable with known protein rich plant foods such as soybean and groundnut. It was found that the different processing methods applied in this study changed the nutrient content of the seeds. Since bean seeds for human consumption will be processed before use, a more elaborate study to optimize the processing methods of black turtle bean is required before an appropriate processing method can be recommended for this important underutilized food crop.

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