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Effects of Processing on Physicochemical and Antinutritional Properties of Black Turtle Bean (*Phaseolus vulgaris* L.) Seeds Flour

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ABSTRACT

The black turtle bean (*Phaseolus vulgaris* L.) is popular among the people of Bokkos in Plateau State, Nigeria where it is cultivated and locally known as "Kwakil". The effects of different processing methods (boiling, cooking, roasting, sprouting and fermenting) were investigated on fatty acid composition, physicochemical parameters and antinutritional factors of black turtle bean seeds. All the analyses were carried out using standard analytical techniques. The results showed that oleic acid and linoleic acid were the most concentrated fatty acids in the raw and processed samples with values ranging from 27.7 to 30.8% and 54.7 to 64.0%, respectively. Capric, lauric and myristic acids were present in small quantities with maximum value of 1.6% in roasted sample. Unsaturated fatty acids predominated in all the samples with an adequate amount of essential fatty acids. Significant differences were observed ($p<0.05$) in the fatty acid compositions between the raw and processed seed samples. The results of physicochemical properties of the seed oils for all the samples showed mean range values of the following parameters: Saponification value (190.5 – 198.05 mg KOH/g), peroxide value (3.20 – 3.42 meq O_2/kg), iodine value (136.4 – 146.4 mg of I/100g, acid value (10.40 – 10.78 mg KOH/g), kinematic viscosity at 100°C (8.12 – 8.44 mm^2/s), specific gravity at 25°C (0.96 – 0.97), unsaponifiable matter (3.37 – 3.62%) and flash point (302 – 315°C). Generally, the values of the physicochemical parameters showed that the oils may be useful as edible oils due to their stability as frying oils. The results of antinutrients revealed that reductions were almost recorded in all the processed seed samples compared with the raw sample.

Key words: Black turtle beans, processing, fatty acids, physicochemicals, antinutrients.

INTRODUCTION

Recent problems linked to meat consumption have also led to renewed interest in vegetarian diet. This phenomenon is reinforced by the fact that physicians have pointed out that consumers eat too many animal products (rich in saturated fat) and no enough plant foods[1]. The consumption of a great deal of meat increases the risk of cardiovascular diseases and some types of

cancer. Therefore, the utilization of seed flour and plant proteins as functional ingredients in food system continued to be of research interest especially in legumes.

Furthermore, legumes are known to be superior in terms of the provision of protein without cholesterol, fibre and minerals such as calcium, magnesium and potassium[2]. Legumes like other foods for human and animals provide some life sustaining elements. These elements are the nutrients that supply energy after being metabolized in the body and are essential for the body to carry on this metabolism[3]. Despite these useful constituents that are present in these seeds, its utilization has been ignored because it has been established that there are compounds or substances which act to reduce nutrient intake, digestion, absorption and utilization[4]. They include tannins, saponins, phytates, flavonoids, alkaloids, cyanogenic, glycosides, etc. Some workers[5] noted that some tropical seeds are high in antinutrients which limit their utilization in food system. Due to effect of these metabolites resulting in food poisoning, there is need to properly address this problem in Nigeria, and indeed in most parts of the developing countries that feed mostly on legumes and other vegetable based diets. This is because people have died of ignorance, poverty and inadequate nutrition education, especially with the African society.

The objective of the present study was to investigate the efficiency of processing methods, such as boiling, cooking, roasting, sprouting and fermenting on the fatty acid composition and some physicochemical parameters of black turtle been seed oils as well as antinutritional components of the seeds flour with a few to providing preliminary information towards effective utilization of this crop in various food applications in Africa and other parts of the world.

MATERIALS AND METHODS

Sample Collection

Mature seeds of black turtle been (*Phaseolus vulgaris* L.) were purchased in September, 2011 from a local farmer in Bokkos town, Bokkos Local Government Area of Plateau State, Nigeria. The seeds were identified at the Herbarium of the Nutritional institute of Pharmaceutical Research and Development (NIPRID) Idu, Abuja, Nigeria.

Sample Preparation

Raw seeds

About 600 g of the raw seed were soaked in ordinary water and manually dehulled and dried in an oven at a temperature of 50°C.

Sprouted seeds

Some of the raw seeds (100 g) were allowed to germinate using a method described by Giami and Bakebain[6]. The seeds were arranged in layers on the sawdust in a locally woven reed basket, wetted daily and observed for sprouting for a period of 3 – 4 days. Seeds with sprouts about 1 cm long were picked, washed with distilled water, and dehulled, sliced and oven dried at 50°C.

Roasted seeds

One hundred grams of the raw seeds were manually dehulled and roasted on a hot cast iron pan at a temperature of 45 – 55°C. The seeds were continuously stirred until a characteristic brownish color was obtained which indicated complete roasting. The seeds were cooled in desiccators and kept for further analysis.

Fermented seeds

The dehulled seeds (100 g) were wrapped in blanched banana leaves and allowed to ferment for 4 days[6]. The sample was then removed, dried in an oven at a temperature of 50°C till well dried.

Boiled seeds

The raw dehulled seeds were boiled for 45 min in distilled water at 100°C. The boiled seeds were drained using a perforated basket, after which they were dried in an oven at 50°C until well dried.

Cooked seeds

The raw dehulled seeds (100 g) were cooked with distilled water using an aluminium pot for 90 min. It was then poured into a perforated basket to drain the water. It was later dried at a temperature of 50°C in an oven.

After all processing treatments were

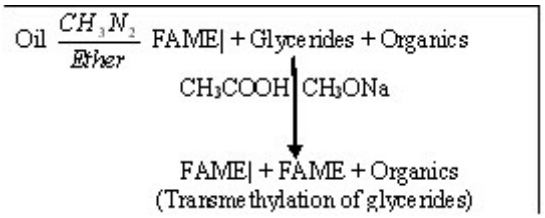
completed all the raw and processed seed samples were ground into fine powder using a Kenwood blender. The flour samples were then kept in airtight containers and stored in a deep freezer (–18°C) prior to chemical analyses.

Extraction of oils

The dried sample was extracted in Soxhlet apparatus with redistilled n–hexane of Analar grade (BDH, London) for the recovery of undiluted oil. The crude oil extract was made to be free of water by filtering through the anhydrous sodium sulphate salt. The hexane was removed from the oil/hexane mixture by sung a rotary evaporator.

Fatty acid analysis

The oil extracted was converted to the methyl ester[8]. About 2 mg crude oil sample was transferred into a 5 – 10 ml glass vial and 1 ml of diazomethane ether solution added. The mixture was shaken thoroughly and allowed to stand for 1 min. then 16 µL of 3.33M CH_3COONa/CH_3OH solution was added; mixture shaken and allowed to stand for 10 min after which 10 µL acetyl acid was added. The equation of the transmethylation is shown below:



The fatty acid methyl esters were analyzed using a HP 6890 gas chromatograph powered with HP Chemistation Rev. a 09.01(1206) software fitted with a flame ionization detector and a computing integrator. Nitrogen was used as the carrier gas. The column initial temperature was 250°C rising at 5°C/min to a final temperature of 310°C while the injection port and the detector were maintained at 310°C and 350°C, respectively. a polar (HP INNO Wax) capillary column (30 m x 0.53 mm x 0.25 µm) was used to separate the esters. The peaks were identified by comparison with standard fatty acid methyl (St. Louis, MO, USA)

Physicochemical analyses

The physicochemical analyses of the oils for saponification value, peroxide value, iodine value, acid value, kinetic viscosity, specific gravity, unsaponifiable matter, and flash point were carried out according to the methods of AOAC[9].

Antinutritional factors determination

The contents of saponin, tannin, alkaloid, trypsin inhibitor, phytate, oxalate and cyanide were determined by methods described by some workers[10-11].

Statistical evaluation

The statistical calculations included percentage value, grand mean, standard deviation and relative standard deviation (RSD).

RESULTS AND DISCUSSION

Fatty acid Composition of Black Turtle Bean

The fatty acid composition of raw and processed seeds of processed black turtle bean is shown on Table 1. Linoleic acid (C18:2É6) had the highest concentration with values ranging from 54.7% in roasted to 64.0% in raw samples with an average value of 60.4±3.48%, oleic acid (C18:1É9) is second with values ranging from 27.7% in sprouted to 30.8% in roasted with average of 29.3±1.19% and palmitic acid (C16:0) is in the third position with values ranging 4.5% in fermented seed to 6.5% in roasted seeds, with average of 5.6%±0.90. The observation in this report is in agreement with reports of some workers as follows: That linoleic (C18:2ω6) was the most concentrated fatty acid in pinto bean (62.9%)[12], soybean (52.0%)[13], red kidney bean (50.3%)[5], corn oil (55.7%), safflower oil (72.6%)[14], and sponge luffa oils (54.32%)[15], but slightly higher than that reported for small black variety of kidney bean (44.3%), cowpea (39.3%) and lima bean (41.0%)[16]. Many lipids from legume seeds contain substantial amounts of saturated fatty acids, especially palmitic acid[17]. A high proportion of either linoleic or linoleic acid is associated with legumes containing insignificant amount of lipids[18]. The lauric acid (C12:0) had the least values ranging from 0.3% in raw to 1.1% in roasted sample with mean value of 0.6±0.33%. Processing had a great effect. The coefficient of variation (CV%) ranged from 4.06 in oleic acid to 62.50 in capric acid.

Table 2 displays the differences in the fatty acid composition between raw and boiled, between raw and roasted, between raw and sprouted and between raw and fermented samples. Capric (C10:0) and lauric (C12:0) acids recorded increase in the boiled, cooked, roasted and sprouted with a reduction in fermented samples while palmitic (C16:0) and stearic (C18:0) recorded an increase in all the processing methods. Linoleic as one of the unsaturated fatty acid and had the highest decrease in all the processing methods (boiling, cooking, roasting, sprouting and fermenting) of 7.34, 10.17, 14.56, 1.56 and 0.47%, respectively which is in perfect agreement with observation made for red kidney seeds. Oleic acid the second unsaturated fatty acid had an increase ranging from 2.74% in cooked to 5.48% in roasted samples and a reduction of an equivalent value of 5.14% in sprouted and fermented seed samples. The slight changes in boiled, cooked and roasted seeds may be due to the effect of thermal cracking on the fatty acid composition while that of sprouted and fermented seeds may be due to metabolic activity taking place in the seeds[19]. CV% was variously varied with a range of 26.15 in oleic acid to 122.50% in capric acid.

The distribution of results in Table 1 into TSFA, MUFA, DUFA, TUFA, TEFA and oleic to linoleic (O/L) is shown on Table 3. TSFA ranged from 8.6% in fermented to 13.9% in roasted samples with an average of 10.32±2.35% and coefficient of variation of 22.77%. These values are lower than TSFA mean value (31.3%) of pigeon pea[15] and Hausa melon seed (28.9%)[21]. Total unsaturated fatty acids (TUFA) which make ω–9 and ω–6 fatty acids ranged from 85.5 to 91.4%. The É–3 and É–6 fatty acids in the diet can be of considerable importance[20] but É–3 fatty acid was not present in all the samples. However, the high content of TUFA in this study is great concern because it has been reported that fats and oils which contain more unsaturated fatty acids are particularly susceptible to oxidation, and intake of food containing oxidized lipids increased the concentration of secondary proxidation products in the liver[21]. Linoleic and α–linolenic acids are the most important essential fatty acids required for growth, physiological functions and body maintenance[17]. Linoleic acid is the only available essential fatty acid in this study ranging from 54.7% in roasted to 63.7% in fermented samples; therefore the raw and processed seeds of black turtle bean will participate well in those functions. It has also been reported that polyunsaturated linoleic acid moderately reduced serum cholesterol and low density lipoprotein (LDL) levels[21]. The oleic/linoleic (O/L) acids ratio has been associated with high stability and potentiality of the oil for deep frying fat[23]. The O/L levels in the present present study ranging from 0.4 to 0.5 are lower than peanut oil (1.48)[23]. Hence black turtle bean oil may also not be useful as frying oil. The CV% ranged from 2.86 in TUFA to56.64% in TEFA%.

Physicochemical Properties of Black Turtle Bean Oil

The physicochemical property of black turtle bean oil is presented on Table 5. The saponification value obtained for the raw black turtle seed oil (190.50 mgKOH/g) is higher when

Table 1: Fatty acid composition (%) of raw and processed black turtle bean seed flour

Fatty acid	Raw I	Boiled II	Cooked III	Roasted IV	Sprouted V	Fermented VI	Mean	RSD	CV%
Capric acid (C10:0)	0.4	0.7	1.4	1.6	0.5	0.3	0.8	0.50	62.50
Lauric acid (C12:0)	0.3	0.5	0.9	1.1	0.4	0.2	0.6	0.33	55.00
Myristic acid (C14:0)	0.9	0.8	1.5	1.2	1.3	0.7	1.1	0.29	26.36
Palmitic acid (C16:0)	4.2	6.1	6.4	6.5	5.7	4.5	5.6	0.90	16.07
Stearic acid (C18:0)	1.1	2.5	2.4	3.5	1.4	2.9	2.3	0.83	36.09
Oleic acid (C18:1ω9)	29.2	30.1	30.0	30.8	27.7	27.7	29.3	1.19	4.06
Linoleic acid (C18:1ω6)	64.0	59.3	57.5	54.7	63.0	63.7	60.4	3.48	5.76

RSD = Relative standard deviation; CV = Coefficient of variation

Table 2: Differences in the fatty acid composition (%) between raw and processed black turtle beans

Fatty Acid	I – II	I – III	I – IV	I – V	I – VI	Mean	RSD	CV%
Capric acid (C10:0)	–0.3(75.00%)	–0.1(–250.00%)	–1.2(–300.00%)	–0.1(–25.00%)	0.1(25.00%)	0.4	0.49	122.50
Lauric acid (C12:0)	–0.2(–66.67%)	–0.6(–200.00%)	–0.8(–266.67%)	–0.1(–33.33%)	0.1(33.33%)	0.4	0.29	72.50
Myristic acid (C14:0)	0.1(11.11%)	–0.6(–66.67%)	–0.3(–33.33%)	–0.4(–44.44%)	0.2(22.22%)	0.3	0.17	56.67
Palmitic acid (C16:0)	–1.9(–45.24%)	–2.2(–52.38%)	–2.3(–54.76%)	–1.5(–35.71%)	–0.3(–7.14%)	1.6	0.73	45.63
Stearic acid (C18:0)	–1.4(–127.27%)	–1.3(–118.18%)	–2.4(–218.18%)	–0.3(–27.27%)	–1.8(–163.64%)	1.4	0.69	49.29
Oleic acid (C18:1ω9)	–0.9(–3.08%)	–0.8(–2.74%)	–1.6(–5.48%)	1.5(5.14%)	1.5(5.14%)	1.3	0.34	26.15
Linoleic acid (C18:1 ω6)	4.7(7.34%)	6.5(10.17%)	9.3(14.53%)	1.0(1.56%)	0.3(0.47%)	4.4	3.37	75.91

I = Raw; II = Boiled; III = Cooked; IV = Roasted; V = Sprouted; VI = Fermented; RSD = Relative standard deviation; CV = Coefficient of variation

Table 3: Fatty acid distribution according to saturation and unsaturation of components (%) in raw and processed black turtle bean

Fatty Acid	RawI	BoiledII	CookedIII	RoastedIV	SproutedV	FermentedVI	Mean	RSD	CV%
TSFA	6.9	10.6	12.5	13.9	9.4	8.6	10.3	2.35	22.77
TSFA (%)	6.9	10.6	12.5	13.9	9.4	8.6	10.3	2.35	22.77
MUFA	29.2	30.1	30.0	30.8	27.7	27.7	29.3	1.17	4.07
DUFA	64.0	59.3	57.5	54.7	63.0	63.7	60.4	3.48	5.77
TUFA	93.2	89.4	87.5	85.5	90.7	91.4	89.6	2.54	2.83
TUFA (%)	93.1	89.4	87.5	85.5	90.7	91.4	89.6	2.54	2.83
TEFA (%)	63.9	59.3	57.4	55.0	63.0	63.7	60.4	3.40	56.64
TNEFA (%)	36.1	40.7	42.6	44.7	37.0	36.3	39.6	3.32	8.39
O/L ratio	0.46	0.51	0.52	0.56	0.44	0.43	0.4	0.08	19.34

TSFA = Total saturated fatty acid; MUFA = Monounsaturated fatty acid; DUFA = Diunsaturated fatty acid; TUFA = Total unsaturated fatty acid; TEFA = Total essential fatty acid; TNEFA = Total non–essential fatty acid; O/L =Oleic/linoleic ratio; RSD = Relative standard deviation; CV = Coefficient of variation

compared with the 106.6 mgKOH/g for *Persea gratesima*[24] and 108.27 mgKOH/g for *Luffa cylindrical*[15]. All the processing methods enhanced the saponification values ranging from 192.50 mgKOH/g in boiled sample to 198.02 mgKOH/g in roasted sample. These high values indicate that the oil contain high proportion of lower fatty acids[25].

The peroxide values (3.20–3.40 meq/kg oil) are higher than those from Africa walnuts oil (1.42 and 1.99 meq/kg oil) *Prosopis africana* oil (1.61 meq/kg oil) but lower than 20 me/qkg for *Luffa cylindrical*[14]. Peroxide value is an indication of deterioration of oil[26]. The peroxide value of 3.2 meq/kg placed the oil within the stipulated permitted maximum level of not more than 10 meq/ kg oil. This suggests that it is not highly susceptible to lipid oxidation. Iodine value indicates extent of unsaturation of the fatty acids present in the fat. The value in this report is higher than 100 g/100g expected maximum level therefore the oil would not be stable as drying oil. All the processing methods reduced the iodine value. The trend is such that raw > boiled > fermented > cooked > sprouted > roasted. The acid value compared well with the value obtained for African walnut oil (11.95 and 12.67 mg KOH/g).The value 10.02 mgKOH/g of the oil is higher than the reported values of 1.96% for *Afzelia africana* seed oil, 3.36% African pear, 2.96% paprispka seed oil, 3.90-4.41%. *Tetracarpidinm conophorum* oil, 3.99-4.10 of raw linseed oil, 1.6% perilla oil but lower than 35.46% African star apple (*Chysophyllum africana*), 13.40 of Horse eye bean and 14% of *Velvet tainarind*[27-29]. The specific gravity of 0.959 obtained indicates that the oil is less dense than water. The value obtained for the raw was 3.92 meq/kg. The value was lower than values for Nigeria palm kernel (1.42 and 1.19 meq/kg). However, the values are still within expected values indicating that the oil can be kept for sometime without much deterioiation (that is it is not highly susceptible to lipid oxidation). The value of 3.92 meq/kg oil falls within the stipulated permitted maximum level of not more than 10 meq/kg oil.

The iodine value is the measure of the extent of unsaturation in the oil. It also helps to place the oil as stable, non-drying oil. The iodine value in this study ranged from 123.48 unit in sprouted sample to 134.25 mgI/g in boiled sample. The values are higher than those reported for red kidney bean (89.4mg iodine/g)[5], *Proposis africana* (59.94 g/100g)[29] but lower than those for *Citrullus vulgaris* (38.1 ± 3%)[29] and Hausa melon seed (38.50 ± 0.67%)[32] Since drying oils have iodine value above 100[31]. Black turtle bean oil can be regarded as drying oil.

Antinutritional Composition of Black Turtle Bean Seeds

Table 8 presents the anti nutrients of the black turtle bean. The saponin content ranged from 2.50% in boiled sample to 13.00% in fermented sample. The value for the raw (13.20%) compared well with values for pinto (13.00%) and red kidney (14.00%) in this work but have higher value than the ones reported by many authors such as Sesbania seeds (0.50–1.46%)[35], 0.05 and 0.23 reported for mung beans and chickpeas, respectively[35]. Saponin has been shown to possess both deleterious properties and to exhibit structure dependent biological activities[33]. The level of tannin in the black turtle seed ranged from 111.75% in the boiled seeds to 124.70% in the cooked, roasted, sprouted and fermented samples. The value is extremely higher than values reported for *Sesbania* seeds (1.97–2.25%)[34].

The nutritional effects of tannins are mainly related to their interaction with protein due to the formation of complexes[34]. Tannin–protein complexes are insoluble and protein digestibility is decreased[35]. Tannin acid may decrease protein quality by decreasing digestibility and palatability. Other nutritional effects which have been attributed to tannin include damage to the intestinal tract, interference with the absorption of iron and a possible carcinogenic effect[36]. The alkaloid content ranged from 1.00% in fermented to 1.80% in boiled sample. Alkaloids cause gastrointestinal and neurological disorders. The values in this research are within the limit since it is below 20 mg/100g which is the limit. Phytic acid content ranged from 37.50 mg/g in the sprouted to 112.50 mg/g in boiled. The 112.50 mg/g obtained for the raw sample is higher than values reported for soybean (40.5 mg/g), pigeon pea (11.7 mg/g) and cowpea (20.4 mg/g)[37]. Phytic acid is an important storage form of phosphorus in plant, it is insoluble and cannot be absorbed in human intestine and it has 12

Table 4: Differences in the distribution according to saturation and unsaturation of components (%) between raw and processed black turtle beans

Fatty Acid	I – II	I – III	I – IV	I – V	I – VI	Mean	RSD	CV%
TSFA	-3.7(-53.62%)	-5.6(-81.16%)	-7.0(-101.43%)	-2.5(-36.23%)	-1.7(-26.64%)	-4.1	2.19	-53.35
TSFA (%)	-3.7(-53.62%)	-5.6(-81.16%)	-7.0(-101.43%)	-2.5(-36.23%)	-1.7(-26.64%)	-4.1	2.19	-53.35
MUFA	-0.9(-3.08%)	-0.8(-2.73%)	-1.6(-5.48%)	1.5(5.14%)	1.5(5.14%)	-0.06	1.45	-2428.42
DUFA	4.7(7.34%)	6.5(10.16%)	9.3(14.31%)	1.0(1.56%)	0.3(0.47%)	4.36	3.77	86.48
TUFA	3.8(4.07%)	5.7(6.12%)	7.7(8.26%)	2.5(2.68%)	1.8(1.93%)	4.3	2.41	56.08
TUFA (%)	3.8(4.07%)	5.7(6.12%)	7.7(8.26%)	2.5(2.68%)	1.8(1.93%)	4.3	2.41	56.08
TEFA (%)	4.6(7.20%)	6.5(10.17%)	8.9(13.93%)	0.9(1.41%)	0.2(0.31%)	4.22	3.69	87.41
TNEFA (%)	-2.9(-7.26%)	-1.5(-3.73%)	-2.4(-5.94%)	-0.5(-1.18%)	-0.9(-2.20%)	-1.64	1.00	-61.22
O/L ratio	-0.02(-4.00%)	-0.01(-2.00%)	-0.05(-10.00%)	0.01(2.00%)	-0.02(-4.00%)	-0.018	0.02	-120.44

TSFA = Total saturated fatty acid; MUFA = Monounsaturated fatty acid; DUFA = Diunsaturated fatty acid; TUFA = Total unsaturated fatty acid; TEFA = Total essential fatty acid; TNEFA = Total non-essential fatty acid; O/L =Oleic/linoleic ratio; I = Raw; II = Boiled; III = Cooked; IV = Roasted; V = Sprouted; VI = Fermented; RSD = Relative standard deviation; CV = Coefficient of variation

Table 5: Physicochemical properties of the raw and processed black turtle bean

Parameter	I	II	III	IV	V	VI	Mean	RSD	CV%
Saponification mgKOH/g of oil	190.5	192.6	195.28	198.05	196.83	194.16	194.57	2.77	1.42
Peroxide value meq/kg	3.2	3.22	3.37	3.42	3.4	3.31	3.32	0.093	2.81
Iodine Value g/100g	146.4	142.34	139.5	136.4	138.42	140.41	140.58	3.47	2.47
Acid Value mgKOH/g of oil	10.4	10.46	10.65	10.78	10.7	10.53	10.52	0.27	2.59
Kinematic Viscosity (mm^2/s) @ 100	8.12	8.28	8.42	8.5	8.44	8.32	8.35	0.14	1.6
Specific Gravity (g/cm^3)	0.959	0.961	0.966	0.972	0.97	0.964	0.97	0.005	0.52
Unsaponifiable Matter (%)	3.62	3.59	3.5	3.37	3.48	3.55	3.85	0.81	21.09
Flash Point()	315.0	313.0	309.0	302.0	305.0	311.0	309.17	4.92	1.59

I = Raw; II = Boiled; III = Cooked; IV = Roasted; V = Sprouted; VI = Fermented; RSD = Relative standard deviation; CV = Coefficient of Variation

Table 6: Difference in the Physicochemical Properties of the Raw and Processed black turtle Bean

Parameter	II	III	IV	V	VI	Mean	RSD	CV%
Saponification mgKOH/g of oil	-4.78(-2.51)	-4.78(-2.51)	-7.55(-3.96)	-6.33(-3.32)	-3.66(-1.92)	-4.88	2.15	-43.98
Peroxide value meq/kg	-0.170(-5.31)	-0.17(-5.31)	-0.22(-6.87)	-0.20(-6.25)	-0.11(-3.44)	-0.14	0.08	-56.12
Iodine Value g/100g	6.90(4.71)	6.90(4.71)	10.0(6.83)	7.98(5.45)	5.99(4.09)	6.99	2.21	31.60
Acid Value mgKOH/g of oil	-0.25(-2.40)	-0.25(-2.4)	-0.38(-3.65)	-0.30(-2.88)	-0.13(-1.25)	-0.60	0.13	-21.35
Kinematic Viscosity (mm^2/s) @ 100°C	-0.30(-3.69)	-0.30(-3.69)	-0.38(-4.68)	-0.32(-3.94)	-0.20(-2.46)	-0.27	0.090	-33.13
Specific Gravity (g/cm^3)	-0.01(-0.73)	-0.01(-0.73)	-0.01(-1.36)	-0.01(-1.15)	-0.01(-0.52)	-0.01	0.00	-58.55
Unsaponifiable Matter (%)	0.12(3.31)	0.12(3.31)	0.25(6.91)	0.140(3.87)	0.07(1.93)	-0.28	0.89	-323.54
Flash Point(°C)	6.0(1.90)	6.0(1.90)	13.0(4.13)	10.0(3.17)	4.0(1.27)	7.00	4.47	63.89

I = Raw; II = Boiled; III = Cooked; IV = Roasted; V = Sprouted; VI = Fermented; RSD = Relative standard deviation; CV = Coefficient of Variation

Table 8: Anti nutritional properties of the black turtle bean

Parameter	Raw I	BoiledII	CookedIII	RoastedIV	Sprouted V	Fermented VI	Mean	SD	CV%
Saponin %	13.2	7.5	2.5	1.3	11.0	13.0	8.08	5.22	64.61
Tannin %	124.7	111.75	124.71	124.7	124.7	124.7	122.54	5.29	4.31
Alkaliod %	5.0	1.8	1.6	0.8	3.4	1.0	2.27	1.62	71.61
Cyanogenic/Glycoside mol/dm^3	4.64×10^{-5}	4.12×10^{-5}	4.18×10^{-5}	2.58×10^{-5}	3.61×10^{-5}	1.55×10^{-5}	3.447×10^{-5}	1.1654×10^{-5}	33.81
Trypsin inhibitors mg/g	150.0	240.0	180.0	150.0	210.0	120.0	175.0	44.16	25.23
Phytic Acid mg/g	112.5	122.5	62.5	62.5	37.5	62.5	75.0	30.62	40.82
Oxalate mg/g	1668.9	1153.6	593.6	660.8	1108.8	996.5	1030.36	389.24	37.78
Cyanide mg/g	Nd	Nd	Nd	Nd	Nd	Nd	–	–	–

I = Raw; II = Boiled; III = Cooked; IV = Roasted; V = Sprouted; VI = Fermented; SD = Standard Deviation; CV = Coefficient of Variation

Table 9: Difference in the Anti nutritional properties of the black turtle bean

Parameter	I–II	I–III	I–IV	I–V	I–VI	Mean	RSD	CV%
Saponin %	5.7(43.18)	10.7(81.06)	11.89(90.15)	2.19(16.67)	0.20(1.52)	6.14	5.12	83.43
Tannin %	12.95(10.38)	-0.01(-0.01)	0.0(0)	0.0(0)	0.0(0)	2.59	5.79	223.61
Alkaloid %	3.2(64.0)	3.4(68)	4.2(84)	1.6(32)	4.0(80)	3.28	1.03	31.27
Cyanogenic/Glycoside mol/dm^3	0.00(11.21)	0.00(9.91)	0.00(44.4)	1.03×10^{-5} (22.2)	0.00(66.59)	0.0	1.0×10^{-5}	
Trypsin inhibitors mg/g	-90.0(-60)	-30.0(-20)	0.0(0)	-60.0(-40)	30.0(20)	-30.0	47.43	-158.1
Phytic Acid mg/g	-10.0(-8.89)	50.0(44.44)	50.0(44.44)	75.0(66.67)	50.0(44.44)	45.0	27.39	60.86
Oxalate mg/g	515.3(30.88)	1075.30(64.43)	1008.1(60.41)	560.10(33.56)	672.4(40.29)	766.24	258.9	33.80
Cyanide mg/g	Nd	Nd	Nd	Nd	Nd	–	–	–

I = Raw; II = Boiled; III = Cooked; IV = Roasted; V = Sprouted; VI = Fermented; RSD = Standard Deviation; CV = Coefficient of Variation

replacaeble hydrogen atoms with which it could form insoluble salts with metals such as calcium, iron. zinc and magnesium. The formation of these insoluble salts renders the metals unavailable for absorption into the body. Phytate can also affect digestibility by chelating with calcium or by binding with substrate or proteolytic enzyme. Phytate is also associated with cooking time in legumes[38]. Oxalate presented in this work (1668.90 mg/g) is lower than 2257.40 mg/g found in red kidney bean (2257.40 mg/g) and higher than pinto (1481.60 mg/g) in this present work but also higher than 2.54 mg/g for soybean, 2.86 mg/g (pigeon pea)[37].

Oxalate is produced and accumulated in many crop plants and pasture weeds. Oxalate is a concern in legumes because high oxalate diets can increase the risk renal calcium absorption since calcium is made unavailable to the body due to the presence of oxalate[39]. Cyanide was not found in all the food samples. Trypsin inhibitors have been found in legumes to inhibit the activity of digestive enzymes hence causing poor utilization of the protein in most legumes[40]. The value obtained for the raw sample was 150 mg/g and ranged from 120 mg/g in fermented to 240 mg/g in boiled indicating a very high value. Researchers observed that those legumes which had the highest trypsin inhibitor activity were those in which the digestibility, as measured in *in–vivo* with rats, was most improved by cooking. They depress animal growth by interfering with the digestion and absorption of nutrients in the gastrointestinal tract[41]. Cyanogenic glycosides ranged from 0.0000155 mol/dm^3 in fermented sample to 0.0000464 mol/dm^3 in raw sample. The value for the raw 0.0000464 mol/dm^3 is very low but compared to values reported for red kidney and pinto bean seeds. This is known to cause acute or chronic toxicity. Cyanogenic glycoside on hydrolysis yield toxic hydrocyanide acid (HCN). The cyanide ions inhibit several enzyme systems; depress growth through interference with certain essential amino acids and utilization of associated nutrients[42].

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