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Physico Chemical and Fatty Acid Composition of Nicker Bean (*Entada gigas*) Seed Oil

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Abstract The proximate, minerals, physico chemical properties and fatty acid composition of nicker bean (*Entada gigas*) seed oil were evaluated. The results of proximate analysis were: moisture (25.0%), ash (0.50%), crude protein (0.23%), crude fat (60.4%) and carbohydrate (13.9%). The predominant mineral was magnesium (161mg/kg) followed by potassium (110 mg/kg) and sodium took the third position with value of 89.0 mg/kg. The least abundant mineral was phosphorus (5.45 mg/kg). The physico chemical parameters analysed were: saponification value (236mgKOH/g), specific gravity (0.7499 g/cm^3), refractive index (1.4651), acid value (3.37 mgKOH/g), unsaponifiable matter (21.62%), peroxide value (4.00 MEq.O_2/Kg), iodine value (13.96 mgI_2/100g) and viscosity (5.52 mPa/sec). The richest fatty acid was oleic acid with the value of 72.1% while the least was lauric acid (0.11%). The value of TUFA >TSFA makes the oil worthwhile.

Keywords Proximate, Minerals, Physico chemical, Fatty acid, Nicker, Oil

1. Introduction

Some of the under developed countries are dearth of food supply, especially that of protein [1]. Legumes are increasingly used by densely populated regions of the world to circumvent the precarious situation of protein shortage in diets. There is inadequate information on the nutritional parameters of some under utilised legumes. *Entada gigas* is commonly known as sea heart. It is a legume flowering plant of the pea family, Fabaceae. A native of central America, Caribbean and Africa. It measures 12cm (4.7inch) across and can reach 2m (6.6ft) in length. Inside the pods are 10 – 15 seeds, each of which has a diameter of 6cm (2.4inch) and a thickness of 2cm (0.70inch). The seeds contain a hollow cavity which gives them buoyancy and resistance to decay when they are washed by rain into rivers and oceans. The seeds of *Entada gigas* can drift long distance on ocean currents. Many legume species contain nitrogen fixing bacterial inside the root nodules which convert inert atmospheric nitrogen into ammonia, thereby making viral nitrogen available to other plants for nutrients. The pod may be 3 – 6ft long before it breaks apart into 15 or more seeded compartment, each bearing a shining brown sea heart [2]. *Entada gigas* has not been used by people for food due to the fact that it contains toxins which are anti nutrients but it is a very popular seed used in the preparation of traditional concoction for curing diseases in Nigeria. Some similar studies had been done by previous workers on some under utilised legumes such as *Terminalia catappa* [3], kidney bean [4], benniseed [5], *Luffa cylindrica* [6], bambara groundnut [7] and velvet tamarind [8]. The aim of this work is to determine the nutritional potentials of both flour and oil of *Entada gigas*.

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2. Materials and Methods

The *Entada gigas* was obtained from Ado - Ekiti, Ekiti State, South west Nigeria in Africa continent. The seeds were dehulled by breaking the seed coat from the inner seed using hammer. The inner seeds were further removed, dried and milled into flour using Marlex grinder. The flour was packaged in a polythene bag and kept in freezer at -4℃ prior to experimental determinations. The oil used for the analyses was extracted by using soxhlet apparatus [9].

2.1. Proximate Analysis

The moisture and ash contents were determined using the air oven and dry ashing method [9]. The sample was analyzed for crude fat and crude protein according to the method described [10]. Nitrogen was determined by micro-Kjedahl method described [3] and the percentage nitrogen was converted to crude protein by multiplying by 6.25. The crude fibre was determined by adding 2g (W_1) of the sample into 500ml conical flask; 200ml of boiling 1.25% of H_2SO_4 was added and boiled for 30minutes. The mixture was filtered through muslin cloth and rinsed with hot distilled water. The sample was scrapped back into the flask and 200ml of boiling 1.25% NaOH was added and allowed to boil again for another 30 minutes; filtered and then rinsed

with 10% HCl twice with industrial methylated spirit and allowed to drain and dry. The residue was scrapped into a crucible, dried in the oven at 105℃, allowed to cool in a desicator and weighed (W_2); then placed in muffle furnace at 300℃ for 30 minutes and finally allowed to cool at room temperature and weighed again (W_3) [10].

$$\% \text{ crude fibre} = \frac{W_2 - W_3 \times 100}{W_1} \quad (1)$$

The carbohydrate content was calculated by method of difference [11].

$$\%CHO = \{100 - (\% \text{ moisture} + \% \text{ Ash} + \% \text{ crude fibre} + \%\text{Crude fat} + \% \text{ Crude protein})\} \quad (2)$$

2.2. Mineral Analysis

The minerals were analyzed by dry ashing the sample at 550℃ to constant weight and dissolving the ash in 100 ml standard flask using distilled deionized water with 3ml of 3M HCl. Sodium and potassium were determined by using a flame photometer (model 405, corning, U.K). All other minerals were determined by Atomic Absorption Spectrophotometer (Perkin & Elmer model 403, USA) [11].

2.3. Physico Chemical Properties

2.3.1. Determination of Saponification Value

A 2.0ml of the oil sample was added to the 20ml of ethanolic potassium hydroxide in 500ml round bottom flask. The flask with its content was refluxed for 30 minutes. 2ml of phenolphthalein indicator was added and the hot solution was allowed to cool and later titrated against the 0.5M hydrochloric acid. A blank titration was carried out using the same procedure [9, 12].

$$\text{Saponification value} = \frac{56.1N\ (V_1 - V_2)}{W} \quad (3)$$

Where:
N = Normality of hydrochloric acid.
V_1 = volume of HCl used in the test.
V_2 = volume of HCl used in the blank.
W = weight of sample oil.

2.3.2. Determination of Peroxide Value

A 2.0g of the oil sample was weighed into the 200ml conical flask containing 20ml of petroleum ether and heated for 30 seconds in a water bath. 20ml of 50% aqueous solution of potassium iodide and 25ml of distilled water were added. The resulting mixture was titrated with 0.002M sodium thiosulphate solution. During the titration a milky white precipitate was observed and the total disappearance of the precipitate indicated the end point of the titration. The peroxide value of the sample oil was estimated on the basis of the equation below. The same procedure was repeated for the blank [8].

$$\text{Peroxide value} = \frac{100\ (T_B - T_S)}{\text{Weight of sample oil}} \quad \text{MEq } O_2/\text{kg} \quad (4)$$

Where:
N = normality of thiosulphate
T_S = volume of thosulphate used in the sample test.
T_B = volume of thiosulphate used in the blank.

2.3.3. Determination of Acid Value

A 5g of the sample oil was weighed into a 250 ml conical flask. 50 ml of hot neutralized alcohol was measured into the flask. The content in the flask was boiled on a water bath, after which 5 drops of phenolphthalein indicator was added into the content of the flask. The mixture was then titrated with 0.1M sodium hydroxide using a burette until a pink colour was observed, indicating the end point [8].

$$\text{Acid value} = \frac{N \times T_B - T_S}{\text{Weight of sample oil}} \quad (5)$$

Where; N = normality of sodium hydroxide.
T_S = Titre value of the sample.
T_B = Titre value of the blank

2.3.4. Determination of Iodine Value

0.2g of the sample oil was transferred into a flask containing 10ml carbon tetrachloride. 25ml of Wijs solution was added into the flask containing the sample (Wijs solution consists of iodine monochloride in glacial acetic acid). Blank was prepared. The mixture was stored in a dark place for 30 minutes at temperature of 25℃ after which 15ml potassium iodine solution was added along with 100ml of distilled water. The resulting mixture was titrated with 0.1M sodium thiosulphate solution using 2ml of 1% starch indicator. The titration was continued until the blue colour just disappeared, indicating the end point [8, 9].

The iodine value was calculated on the basis of the following equation:

$$\text{Iodine value} = \frac{12.692\ (T_B - T_S) \times N}{\text{Weight of the sample oil}} \quad (6)$$

Where; N = normality of the solution.
T_S = Titre value of the sample.
T_B = Titre value of the blank

2.3.5. Determination of Unsaponifiable Matter

After saponification, 300ml of the mixed solvent of ethanol (70%), toluene (25%) and 5ml oil was added to the packed glass column. It was allowed to run through the column at the rate of 12ml / minute. The glass column was washed with 150ml of the solvent mixture at the same rate. It was concentrated to 25ml using rotary evaporator and then transferred to the tarred dish for evaporation in oven at 105°C for 15 minutes. The dried sample was weighed and titrated for the remaining acids; the weight was corrected for the unsaponifiable matter [10].

2.3.6. Determination of Specific Gravity

The sample (40ml) was homogenized and poured into a 500ml measuring cylinder gently to avoid air bubbles. The temperature was controlled to avoid drifting in the temperature value. Hydrometer was dipped into the oil carefully to avoid resting on the wall of the cylinder and the reading was then taken [8].

2.3.7. Determination of Refractive Index

The oil was dried to make it free of moisture. Two drops of the oil was put on the lower prism of the equipment and the prism was closed up. The water was passed through the jacket at 45℃, the jacket was adjusted until the equipment read temperature of 40℃. The light was adjusted and the compensator was moved until a dark border line was observed on the cross wire. The reading on the equipment was recorded [8].

2.3.8. Determination of Kinematic Viscosity

A viscometer was employed for the determination. The water bath was maintained at the operating temperature by thermo regulator. The wide capillary viscometer tube was selected for the analysis. The sample was filtered through the no 200 mesh sieve to remove the associated solid particles. Sample was introduced into the viscometer in a manner stated by the equipment manual. The charged viscometer was allowed to stay in the water bath long enough to reach the test temperature. Suction was used to adjust the head level of the test sample to a position in the capillary arm of the first timing mark. As the sample was flowing freely, measurement was done to 0.2 second and the time required for the meniscus to pass from the first time mark to the second was noted. The kinematic viscosity was calculated based on equation 7 below [3].

$$V = CT \quad (7)$$

V = Kinematic viscosity
C = calibration Constant
T= Flow time in seconds

2.4. Fatty Acid Profile

The fatty acid profile was determined using a method described [13]. The fatty esters analyzed using a PYE Unicam 304 gas chromatography fitted with a flame ionization detector and PYE Unicam computing integrator. Helium was used as carrier gas. The column initial temperature was 150℃ rising at 5℃ min^{-1} to a final temperature of 200℃ respectively. The peaks were identified by comparison with those of standard fatty acid methyl esters.

3. Results and Discussion

Table 1 shows the result of the proximate composition of *Entada gigas* seed flour. The moisture content was 25.0%. This value was higher than those of quinoa flour (11.02%) reported [12], ginger bread plum seed flour (10.00%) reported [14] and velvet tamarind (8.22%) [15] but lower than stem (67.2%), root (60.7%) and leaves (75.8%) of *Moringa oleifera* [16]. The crude protein content (0.23%) was lower than those cereal - millet (11.4%) reported [17], ginger bread plum seed flour [14], raw African mango seed (10.6%) [11] and *Luffa cylindrica* (43.1%) [6]. The crude fat of *Entada gigas* seed (60.4%) was higher than those of ginger bread plum seed (47.28%) reported [14], bambara groundnut (6.72%) reported [7], pearl millet (7.6%) [17], *T. Occidentalis* (54.4%) [18], benniseed (44.3%) and bulma cotton seed (47.8%) [5]. This result indicates that *Entada gigas* seed can be grouped as an oil-rich leguminous seed since the oil yield was 60.4%. The ash content (0.50%) was low. This value was lower than those of quinoa flour (1.2%) reported [12] and *Terminalia cattapa* (4.2%) [3]. While the carbohydrate content (13.9%) was lower than those of quinoa flour (58.3%) [12], *Terminalia catappa* (16.02%) [3] and kidney bean (40.0%) [4] but comparable with that of *Luffa cylindrica* (13.6%) [6]. Since the oil was high, therefore, the carbohydrate level would be very low.

Table 1. Proximate conposition of *Entada gigas* seed flour

COMPONENT	%
Moisture	25.0
Ash	0.50
Crude protein	0.23
Crude fat	60.4
Carbohydrate	13.9

Table 2 shows the result of mineral composition of *Entada gigas* seed flour. The highest mineral was magnesium (161 mg/kg) followed by sodium (110 mg/kg).The value of sodium was higher than those of *Terminalia catappa* (27.89 mg/100g) reported [3], quinoa flour (93.0mg/kg) [12] and sorghum flour (61.8 mg/kg) [19]. The value of potassium (89.00mg/kg) in the sample was lower than those of defatted ginger bread plum seed flour (125mg/kg), raw corn flour [20] and quinoa flour (714.0mg/100g) [12]. The magnesium value of the sample was lower than those of *Terminalia catappa* seed (798.6mg/100g) [3] while calcium value of the seed of *Entada gigas* (14.0mg/kg) was lower than those of *Terminalia catappa* seed (827.20mg/100g) reported [3] and unprocessed *V. anygdalina* (97 mg/kg) [21]. The low calcium content of the seed makes it a potential source of calcium supplementation for pregnant, lactating women, children and elderly people [14].The phosphorous content was lower than those of quinoa flour (22.0mg/100g) [12] and unprocessed *V. cororata* (80 mg/kg), *V. calroana Var* bitter (61 mg/kg) [21].

Table 3 shows the results of physico chemical properties of *Entada gigas* oil. The iodine value (14.0 mgI_2/100g) was lower than those of *Sesame indicum* oil white (103 mgI_2/100g) and red (116mgI_2/100g) [14]. The oils with iodine value less than 100 are non-drying oils, therefore, the low iodine value of the sample seed oil indicates that the oil

is a non drying oil and does not make it suitable for making paints and vanishes [22]. The iodine value must not be too high because high value denotes an excessive free fatty acid which causes the oil to turn sour and decolourised. The saponification value of the extracted oil of *Entada gigas* (236mgKOH/g) was higher than those of quinoa oil (192.0%) reported [12], butter fat (220-241%), cotton seed (190-200%) and soy bean (190-194%) [23]. The saponification value of greater than 200mgKOH/g indicates high proportion of unsaturated fatty acids. This shows that the *E-gigas* oil has a very high potential for use in soap making and food industries. The peroxide value (4.00MEqKOH/g) was lower than the maximum acceptable value of 10MEqKOH/g set by the Codex Alimentarius commission for groundnut seed oils [22]. This value was found to be higher than that of quinoa oil (2.44%) [12] .A high peroxide value indicates a poor resistance of the oil to per oxidation during storage. The low value obtained for the sample indicates that the oil would not easily go rancid when properly stored in a container free from atmospheric oxygen and other contaminants. The *Entada gigas* oil had a specific gravity of 0.7499 g/cm^3 at 28℃. This value was slightly lower than *Laphira lanceolata* (0.8869g/cm^3) and *Sclereocararya birrac* (0.8975g/cm^3) [24]. The low specific gravity indicates that the oil is less dense than water.

Table 2. Mineral composition of *Entada gigas* seed flour

MINERALS	mg/kg
Sodium (Na)	110
Potassium (K)	89.0
Magnesium (Mg)	161
Calcium (Ca)	14.0
Phosphorus (P)	5.45

Table 3. Physico chemical properties of *Entada gigas* seed oil

PHYSICOCHEMICAL	VALUE
Acid value (mgKOH/g)	3.37
Free fatty acid (% oleic acid)	1.69
Peroxide value (MEq.O_2/Kg)	4.00
Iodine value (mg I_2/100g)	14.0
Saponification number (mgKOH/g)	236
Unsaponifiable matter	21.6
Specific gravity (g/cm^3)	0.7499
Refractive index (@ 28%,40ºC)	1.4651
Viscosity (mPa/sec)	5.52

Table 4 presents the fatty acid composition of *Entada gigas* oil. The oil is rich in unsaturated fatty acids than the saturated counterpart while the other fatty acids were in trace amount except for palmitic acid which was very low. The highest concentrated fatty acids of the oil were: oleic acid (18:1) and linoleic acid (18:2) with the values of 72.1% and 19.5% respectively. The main unsaturated fatty acids were: linoleic and oleic acids and with these values *Entada gigas* oil can be classified as the oleic – linoleic group. Linoleic acid is one of the most important polyunsaturated fatty acids in human oil foods because of its prevention of distinct heart vascular diseases [25]. Apart from preventing cardiovascular disorders such as coronary heart diseases and atherosclerosis, its presence in the oil also prevents high blood pressure [3]. Therefore, *Entada gigas* oil can reduce the high blood pressure of hypertensive patients whose systolic pressure exceeds 160mmHg or diastolic pressure exceeds 95 mmHg [26]. The oleic acid ($C_{18:1}$) value of the E-gigas seed oil (72.1%) was higher than those of gingerbread plum oil (47.15%) [14], *Terminalia catappa* oil (31.65%) reported [3] and peanut oil (50-65%) [27] but lower than those of olive oil (75%) and almond oil (77%) reported [27]. The oleic / linoleic acid (O/L) ratio of the oil was 3.692, this value was higher than those of ginger bread plum seed flour (2.469%) and *Terminalia cattapa* oil (1.077%) [6]. O/L ratio is an indicator for oil stability. High O/L ratio is associated with high stability and potential of the oil for frying and cooking. It has been established that relative to carbohydrate, the saturated fatty acid elevates serum cholesterol and while unsaturated fatty acid lowers serum cholesterol [28]. So, the oil extracted from the *Entada gigas* contains appreciable amounts of polyunsaturated fatty acid (linoleic acid $C_{18:2}$) with value of 19.5% which may slightly lowers serum cholesterol. Polyunsaturated fatty acids are the most important essential fatty acids required for growth, physiological functions and body maintenance [29]. The high value of oleic acid content makes the oil useful in the production of pharmaceutics, soaps, shampoo and cosmetics. The oil can be used also as; an emollient (protective agent) for softening the skin, irritation alleviator and laxative which promotes defecation [27].

Table 4. Fatty acid composition of *Entada gigas* seed oil

FATTY ACID	%
Myrictic acid (14:0)	0.12
Palmitic acid (16:0)	5.40
Stearic acid (18:0)	1.82
Arachidic acid (20:0)	0.32
Palmitoleic acid (16:1)	0.32
Oleic acid (18:0)	72.1
Linolenic acid (18:3)	0.13
Lauric acid (12:0)	0.11
Linoleic acid (18:2)	19.5
Behenic acid (22:0)	0.30
Lignoceric acid (24:0)	0.12

4. Conclusions

From the results of the analyses, it can be concluded that *Entada gigas* seed has a high minerals density which are

essential ingredients for body development. The high oil yield and high degree of unsaturation qualify its usefulness in the industrial manufacture of pharmaceutics, soaps and cosmetics. Though the toxicity level was not reported in this paper but previous investigations revealed that the sample contains some toxins which hinder people from consuming it.

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