

Physio-Chemical Studies of *Isoberliniadoka* Seedoil for Possible use as Edible Oil

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Abstract: Crude oil extracted from Isoberliniadoka seed, collected from a farm land located in Girel Local Government Area of Adamawa State was investigated for possible use as edible oil. Extraction of the oil from the seed was carried out by Soxhlet method using petroleum ether as the extractant. The physio-chemical properties of the oil were determined using Gas Chromatography (GC) and other standard methods. The result of the physical analysis showed that the seed had a percentage oil yield of 2.2%. The refractive index of the oil was 1.4. The chemical analysis showed that the saponification value was 89.7mgKOH/g and free fatty acid value was 19.3%. The acid value was 38.6mgKOH/g and the iodine value was $1.1gI_2/g$. The gas Chromatography analysis gave the following fatty acid profile: Lauric (9.6%), Myristic (42.7%), Palmitic (23.6%), Hexadecadienoic (0.2%). Stearic (5.4%), Linoleic (12.8%) and Arachidic (39.8%) respectively. The study revealed that the Isoberliniadoka seed is not a good source of edible oil because of its high content of saturated fatty acids. It was also found that the seed oil was not suitable for food formulation as well as pharmaceutical, paints, soap and perfume industries because of its low level of unsaturation in the fatty acid content.

Keywords: Isoberlinedoka, seed oil, physiochemical, edible, studies.

1. INTRODUCTION

Plant seeds are important sources of oils of nutritional, industrial and pharmaceutical importance (Alvarez *et al.*, 2000). Oil seeds can serve as high quality dietary sources to meet nutritional requirements. One of the least expensive ways of increasing protein levels in the diets of low income families is by encouraging the consumption of local indigenous edible seeds especially oil seeds and legumes which have been found to be rich in protein (Singh et al., 1993).

The suitability of oil for a particular purpose, however, is determined by its characteristics and fatty acid composition. No oil from any single source has been found to be suitable for all purposes, as oils from different sources generally differ in their fatty acid composition. The world production of fatty acids (FAs) from the hydrolysis of natural fats and oils totals about 4 million metric tons per year (Minzangi*et al.*, 2011).

Fatty acids (FAs) are utilized in a wide variety of end-use industries that include food, medicine, rubber, plastics, detergents, and cosmetics. Fats and oils make up the greatest proportion of raw materials in the chemical industry (Biermann *et al.*, 2000). However, the sources of oils and fats are diminishing, implying that there is the growing need for new sources of oil to supplement the existing ones (Mohammed *et al.*, 2003).

Isoberliniadoka is a tree native to northern Nigeria and known as doka (Hausa). It grows to about 20m high, with spreading crown and thrives in both temperate and tropical areas. The tree has been used by traditional medical practitioners for the treatment of diabetes, ulcer, wounds and cough. Phytochemical screening of the stem bark extract revealed the presence of saponins, flavonoids,

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alkaloids and volatile oils; the extract was found to exhibits antibacterial activity against *Bacillus subtilis* (Kubmarawa*et al.*, 2007). In spite of the general use of *Isoberliniadoka* as a medicinal plant, there are limited or no studies that investigated the chemical studies, nutritionals (carbohydrate, protein, fat and oil, minerals, vitamins, water) and antinutritional (phytate, oxylate, hydrocyanic acids, alkaloids) content of the seed oil for possible consumption.

Therefore, the present study aimed at investigating the physicochemical, nutritional and antinutritional properties of the seed oil of *Isoberliniadoka* for possible use as edible oil.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

The sample was obtained from a farm land located in Girei Local Government Area, Adamawa State, Nigeria and identified by a Botanist from ModibboAdama University of Technology, Yola.

The seeds were sun dried for two weeks before drying at 105oC till constant weight in an oven. After drying, the seeds were shelled by hand to remove the kernels which were crushed using a coffee-mill to produce fine seed flour from which oil samples were extracted.

2.2 Extraction of Oil from Plant Seed

Oil from the flour was extracted using the Soxhlet's procedure (Barthet et al., 2002), by repeated washing with petroleum ether (boiling point 40 to 60° C). After 8hrs, the Soxhlet extraction flask containing oil and solvent mixture were removed from Soxhlet apparatus. The oil dissolved in petroleum ether was filtered using filter paper (Whatman No. 1) and the solvent evaporated under vacuum in a rotary evaporator. The remaining solvent traces was removed by heating the flask containing oil in a water bath at 90°C. The oil obtained, was stored in closed bottles and kept in a refrigerator at 4°C till further analyses.

2.3 Physicochemical Properties Determination

2.3.1. Analysis of fatty acid (FA)

The FA composition of oil was determined by converting the oil into FA methyl ester (FAME) followed by gas chromatography (GC) as described by (Grahi-Nielsen *et al.*, 2000)

2.3.2. Determination of Acid Value

The acid value is defined as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in one gram of fat. It is a relative measure of rancidity as free fatty acids are normally formed during decomposition of oil glycerides. The value is also expressed as percent of free fatty acids calculated as oleic acid (Fassi, 2012).

The acid value is determined by directly titrating the oil in an alcoholic medium against standard potassium hydroxide/Sodium hydroxide Solution (Fassi, 2012).

The oil sample (1.0g) was weighed and dissolved with 50ml of ethanol in a conical flask. Two drops of phenolphthalein indicator were added and titrated to pink end point (which persisted for 15 minutes) with 0.1N potassium hydroxide solution (KOH). Acid value was calculated as in equation 1 (Fassi, 2012).

Acid Value = 56.1VXC/m

Where; 56.1 = the equivalent weight of KOH

V = Volume in mol of standard volumetric potassium hydroxide or sodium hydroxide used.

- C = Exact concentration in potassium hydroxide solution used (0.1N); and
- m = the mass in gram of the test portion (1g)

The acidity is frequently expressed as free fatty acid for which calculation shall be;

Free fatty acids as oleic acid = 28.2VN/W percent by weight.

2.3.3. Determination of Saponification Value

Saponification value was determined according to titrimetric method discussed by Pearson (1981). Two grams of oil sample were weighed into a conical flask and 25ml ethanoic potassium hydroxide was added. The solution was refluxed for 2 hours with time to time shaking. One ml phenolphthalein

was added and titrated with 0.5N hydrochloric acid (HCl). The same process was conducted for blank determination. The value was calculated as:

Saponification Value = $(V0 - V1) \times C \times 56.1 / m$ (Fssai, 2012).

Where; 56.1 = equivalent weight of potassium hydroxide

V0 = Volume in mol of standard hydrochloric acid used for the blank test

V1 = Volume in mol of standard hydrochloric acid solution used for the sample

C = the exact concentration of the standard hydrochloric acid (0.5N) solution; and

m = mass in gram of the oil/fat taken from the test portion (2g)

2.3.4. Determination of Iodine Value

Weigh accurately an appropriate quantity of the dry oil into a 500ml conical flask with glass stopper, to which 25ml of carbon tetrachloride have been added. Mix the content well. The weight of the sample shall be such that there is an excess of 50 to 60 percent of Wiji's solution over that actually needed. Pipette 25ml of Wiji's solution and replace the glass stopper after wetting with potassium iodide solution. Swirl for proper mixing and keep the flasks in dark for half an hour for non-drying and semi-drying oils and one hour for drying oils. Carry out a blank simultaneously. After standing, add 15ml of potassium iodide solution followed by 100ml of recently boiled and cooled water, rinsing in the stopper also. Titrate liberated iodine with standardized sodium thiosulphate solution, using starch as indicator at the end until the blue colour formed disappears after thorough shaking with the stopper on (Fssai, 2012).

Conduct blank determinations in the same manner as test sample but without oil/fat. Slight variations in temperature appreciably affect titre of I2 solution as chloroform has a high coefficient of expansion. It is thus necessary that blanks and determinations are made at the same time.

Iodine Value = 12.69 (B - S) N/W

Where;B = Volume in ml of standard sodium thiosulphate solution required for the blank

S = Volume in ml of standard sodium thiosulphate solution required for the sample

N = Normality of the standard sodium thiosulphate solution

W = Weight in gram of the sample.

2.3.5. Determination of Peroxide Value

Peroxide value was evaluated according to AOCS, 2003. Five grams oil sample were weighed into a conical flask and 30mlof solvent mixture of glacial acetic acid – chloroform in the ratio of 3:2 were added to the oil sample. Half ml saturated potassium iodide (KI) solution was added to the solution and allowed to stand for 1 minute thereafter, 30 ml of distilled water were added and titrated with 0.01N sodium thiosulfate solution using starch indicator until the yellow colour was discharged. A blank was prepared alongside the oil sample. Peroxide value was calculated as:

Peroxide value = 10 X (V1 - V2)/m

Where; V1 = volume of Na2S2O3 for determination of test sample in ml

V2 = volume of Na2S2O3 for determination of blank solution in ml; and

m = mass of test portion in gram (5.0g)

2.3.6. Determination of Specific Gravity

Fill the dry pycnometer with the prepared sample in such a manner to prevent entrapment of air bubbles after removing the cap of the side arm. Insert the stopper, immerse in water bath at 300oC and hold for 30 minutes (Fssai 2012).

Carefully wipe off any oil that has come out of the capillary opening. Remove the bottle from the bath, clean and dry it thoroughly. Remove the cap of the side arm and quickly weigh, ensuring that the temperature does not fall below 30oC.

Specific gravity = A - B/C - B

Where; A = weight in gram of specific gravity bottle with oil at 30oC

B = Weight in gram of specific gravity bottle at 30oC

C = Weight in gram of specific gravity bottle with water at 30oC

2.3.7. Determination of the Refractive Index

Measurement of refractive index of the sample is done by means of a suitable refractometer.

Melt the sample if it is not already liquid and filter through a filter paper to remove impurities and traces of moisture. Make sure sample is completely dry.

Circulate stream of water through the instrument. Adjust the temperature of the refractometer to the desired temperature. Ensure that the prisms are clean and dry. Place a few drops of the sample on the prism, close the prisms and allow standing for 1 - 2 min. adjust the instrument and lighting to obtain the most distinct reading possible and determining the refractive index (Fssai, 2012).

2.4 Nutrients' Content Determination

2.4.1. Determination of Moisture Content

The moisture content was determined using standard method described by Cocks (1996), 1.0g portion of the sample was weighed into a previously washed, and dried crucible. The sample was dried in an oven at 100-1100C for 2 hours, removed, and cooled in a desiccator and weighed. This was repeated till a constant weight was obtained. The amount of moisture in the sample was calculated as followed:

% moisture =

Where ab=weight of dish + weight of sample before drying(g)

ac=weight of dish + weight of sample after drying(g)

2.4.2. Determination of Lipid Content

A portion of 2.0g of the sample was weighed into a 250ml conical flask and 50ml of diethyl ether was added, shook and allowed to stand overnight. The mixture was then filtered using gravity filtration set and washed down with the same solvent. The ether was recovered by evaporating on a water bath and dried at 1050C in the oven for 1hour and weighed (Cocks, 1996). %oil

2.4.3. Determination of Ash Content

1.0g of the sample were weighed into a previously clean, dried and weighed crucible dishes, and then ashes in muffle furnace at 10000C for 3hours, they were then removed, cooled in desiccators and weighed (Suzane, 1994). The ash content was obtained as % ash content.

2.4.4. Determination of Crude Protein

The Kjedhal method was used for the determination of crude protein. 1.0g of finely grinded sample was introduced into a 500ml Kjedhal flask. 12.5g of potassium sulphate, 0.5g of copper sulphate (catalyst) and then 25ml of concentrated sulphuric acid were added successively. The flask was first heated gently until frothing ceased, then boiled gently until a clear, blue solution was obtained, and the flask was cooled and the content was diluted to 400ml with distilled water. It then transferred to a 1litre flask and two pieces of granulated zinc (about 1g powder) was added. Flask was connected to a distillation apparatus and 60ml of 50% sodium hydroxide was added through a tap funnel.

The flask was swirled and the content was distilled into a 500ml conical flask containing 25ml of 0.2M sulphuric acid and 2-3 drops of methylated was added. The stem of the condenser was dipped into the diluted acid in the conical flask. The flask was heated at first, when there was no danger of distillate was collected. The tip of condenser was washed with distilled water and the distillation was allowed to continue for about 3minutes and a drop of fresh distillate was tested with neutral litmus paper to be sure of non-alkalinity of the solution. The excess acid in the distillate was then titrated against 0.1M sodium hydroxide. A blank determination was carried out using the same amount of reagents but 0.5g of soluble starch in place of the sample, the crude protein was derived follows:% Nitrogen

Crude protein content =6.25 X %Nitrogen

Where W(mg) = weight of sample

V1(ml) = volume of sodium hydroxide in blank titration

V2(ml) = volume of sodium hydroxide in sample titration

N=normality of acid

2.4.5.Determination of Crude Fibre

Crude fibre is a chemical entity, it is the remnant after plant material has been heated with hot concentrated sulphuric acid, alkali or alcohol or other non-physiological solvent. 1.0g of the sample was boiled in 30ml of 0.3M sulphuric acid for 15minutes, followed by addition of 40ml solution of 1.5M sodium hydroxide and boiling continue for another 15minutes and allowed to cooled.

The mixture was filtered and washed several times with 0.3M hydrochloric acid and water successively. The residue was then dried in an oven at 1050C weighed in a crucible and ashes for 2hours at 10000C in muffle furnace (Suzane, 1994) the percentage crude fibre was calculated thus: % crude fibre

2.4.6.Determination of Carbohydrates

The available carbohydrates content was obtained by difference having estimated all the other proximate composition (Barminas et al; %carbohydrates = 100 - (%moisture + %lipid+%ash + %crude protein + %crude fibre)

2.5. Determination of Anti-nutrients Contents

2.5.1. Determination of Oxalate

Oxalate was determination by standard method described by Day and Underwood (1986). 1g of the sample (dried vegetables) was taken and 75ml of 0.75 H2SO4 was added to the sample and it was carefully stirred intermittently with magnetic stirrer for 1houur and filtered using whatman No1 filter paper. 25ml of filtrate (extract) collected and titrated hot (SO-90C0) against 0.1M KMnO4 solution to the point when a faint pink colour appears and persists for 30sec

2.5.2. Determination of Hydrocyanic

Hydrocyanic acid (HCN) was determined based on the procedure described by AOAC (Association of Analytical Chemistry, 1984). 10g of the sample was put in distillation flask 200ml of distilled water was added and allowed to stand for 3hours. It was then steam distilled and 150ml of distillate was collected in 2.5% NaOH solution, the solution was diluted to 250ml and 100ml liquor was taken. This mixture was then titrated with 0.02M AgNO3 solution to afaint permanent turbidity end point.

2.5.3. Determination of Alkaloids

The gravimetric method of Harbone (1980). Was adopted for this determination. 50ml of the sample was measured and dispersed into 50lm of 10% Acetic acid solution in ethanol. The mixture was shake and allowed to stand for 4hours before filtration. The filtrate was evaporated to ¹/₄ of the original volume, concentrated NH4OH was added drop wise. The sample containing Alkaloids formed precipitate, this was filtered, washed with 1% NH4OH and dried in an oven at 60C0 for 30minutes and weighed.

2.5.4. Determination of Phytate

The Reddy and Love (1999) method was adopted. 4g of the sample was soaked in 100ml of 2% HCL for 5hours and filtered. The filtrate was turned to conical flask and 5ml of 0.3% ammonia thiocynate solution was added. The mixture was then titrated with standard Iron (III) chloride persisting for 5minutes was obtained.

3. RESULTS AND DISCUSSION

3.1. Physicochemical properties of seed oil of Isoberlinia Doka

The research work was conducted to determine the physicochemical properties of the seed of *Isoberlinia Doka* for possible use as edible oil. The quality of the oil was analyzed by evaluating physicochemical properties such as Acid value, free fatty acid, saponification value, peroxide value, iodine value, specific gravity, refractive index and viscosity.

The colour of the oil was light/golden yellow. It is liquid at room temperature. The oil content is of great significance in the determination of the produce for processing. The percentage oil yield in the sample was 2.20% which falls below the range 15-20% estimated by Anthony and Offiong (1998).

The Refractive index (RI) is a parameter that relates to molecular weight, fatty acid chain length, degree of unsaturation and degree of conjugation (Gunstone, 2002). The refractive index of the oil analyzed was 1.467, which falls within the range for many edible oils (Egbekun and Ehieze, 1997; Ilesanmi*et al.*, 1990).

Saponification value is used in checking adulteration. The saponification value recorded for the oil was 89.76mgKOH/g Oil. The relatively low value showed that the oil does not have potential for use in the soap industry (Amoo*et al.*, 2004). Higher Saponification value indicates high proportion of lower fatty acids since saponification value is inversely proportional to the average molecular weight or chain length of the fatty acids (Muhammad *et al.*, 2011). Therefore, the shorter the average chain length (C₄-C₁₂) the higher is the saponification number (Tamzid*et al.*, 2007). The value obtained for *Isoberlinia Doka* oil during this study shows that it contains low amounts of short chain fatty acids (C_{12}), which is lower than the regulation of codex standard permissibility level (CODEX-STAN210-1999) (190-209mgKOH/g). Therefore, it is suggested that, *Isoberliniadoka*oil contains high molecular weight long chain fatty acids hence are unsuitable for soap making and also unsuitable for human nutrition (Akinhanmi and Atasie, 2008).

The acid and free fatty acid (FFA) values are used to indicate the level of rancidity of oils (edibility) and their suitability for use in the paint industry. Acid value is a measure of the free fatty acids in oil. Normally, fatty acids are found in the triglyceride form, however, during processing, the fatty acids may get hydrolyzed into free fatty acid. The higher the acid value found, the higher the free fatty acid which translates into decreased oil quality. The acid value obtained was 38.60mgKOH/g and the free fatty acid value obtained was 19.30% respectively. The oil sample presented in this study shows a higher acid value (38.60mgKOH/g) than the Codex Standard for named vegetable oils. The acceptable levels for all oil samples should be below 0-6mgKOH/g (AOCS Official method Cd 8-53, 2003). The value of the free fatty acid obtained in this study (19.6%) indicates high free fatty acid, since the allowable limit for free fatty acids for edible oils is 1.0-3.0% (Paul and Mittal, 1997). Therefore, the oil is unsuitable for human consumption. The lower the FFA level, the better the quality of the oil for human consumption.

The primary concerns with fatty acid consumption relate to two chronic Diseases – coronary Heart Disease (CHD) and Cancer (Amos-Tautua*et al.*, 2013).

Peroxide value of oil is used as a measurement of the extent to which rancidity reactions have occurred during storage. Other methods are available, but peroxide value is the most widely used. Oils with a high degree of unsaturation are most susceptible to autoxidation. The best test for autoxidation (oxidative rancidity) is the determination of the peroxide value, as peroxides are intermediates in the autoxidation reaction. Autoxidation is a reaction involving oxygen that leads to deterioration of fats and oils which form off-flavours and off-odours. Peroxide value is useful for assessing the extent to which spoilage has occurred.

One of the most important parameters that influence lipid oxidation is the degree of unsaturation of its fatty acids. When double bonds of unsaturated fats are oxidized, peroxides are among the oxidation products formed. High peroxide value is an indicator of oxidation level and, the greater the peroxide value, the more oxidized the oil is.

The peroxide value of the oil sample studied is in agreement with the maximum Codex standard peroxide value (10 meq O_2/Kg) for vegetable oil deterioration. *Isoberliniadoka*seed oil studied has significantly low (0.000771 meq O_2/kg) peroxide value and hence high degree of saturation.

The Iodine value (IV) is a measure of the relative degree of unsaturation in oils. The greater the iodine value, the more the unsaturation and the higher the susceptibility to oxidation. The oil sample analyzed has an iodine value of $1.0787 \text{Gi}_2/\text{g}$ hence has more saturated fatty acids.

Table1. Physicochemical properties of seed oil of Isoberlinia Doka

Parameters	Units	Values
Acid Value	MgKOH/g Oil	38.60

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Free Fatty Acid	%	19.30
Specific Gravity	-	0.8996
Saponification Value	MgKOH/g Oil	89.76
Iodine Value	gI2/g Oil	1.0787
Peroxide Value	meq/kg	0.000771
Refractive Index	-	1.467
Viscosity(28°C)	Pa.s	0.0635
Colour	Tintometer	2.20

3.2. Nutrients Contents of Isoberliniadoka Seed Oil

The result for the nutritional content of *Isoberlinia Doka* seed oil is presented in table 2below. The oil contains high lipid content of 54.24 followed by protein content of 19.27moisture, ash, crude fibre and carbohydrates contents are 1.13, 15.10, 1.20 and 9.05 respectively.

Table2. Nutrients Contents of Isoberliniadoka Seed Oil

Parameters	Content (%)
Moisture	1.13
Ash content	15.10
Lipid	54.24
Crude fibre	1.20
Crude protein	19.27
Carbohydrate	9.05

3.3 Anti-nutrient Contents of Isoberliniadoka Seed Oil

The anti-nutrient content of *Isoberlinia Doka* seed oil is presented in table 6 above. These are compounds that limit the wide use of many plants due to ubiquitous occurrence of them as natural compounds capable of eliciting deleterious effect in man and animals (Kubmarawa*et al.*, 2008). The anti-nutrient factors; oxalate, phytate and alkaloid were present in varying amounts in the oil while HCN is absent.

The value of oxalate was 1.32, the value of alkaloid was 0.33 and phytate was found to be 1.28. Food containing phytate of 1-6% over a long period of time decreases the bio-availability of mineral elements such as Ca, Zn, Mn, Fe and Mg to form complexes that are indigestible, thereby, decreasing the bio-availability of these elements for absorption (Makkar and Beckar, 1998).

 Table3. Anti-nutrient Contents of IsoberliniadokaSeed Oil

Anti-nutrient	content (%)
Oxalate	1.32
Phytate	1.28
Alkaloids	0.33
HCN	Nil

3.4 Composition of fatty acids in the seed oil of Isoberliniadoka

From nutritional viewpoint, the presence of Oleic acid in diet is very useful. It has been shown that Oleic acid is effective in lowering total cholesterol (10%) and Low-Density Lipoprotein (LDL) cholesterol content in blood (Grundy, 1999; Dennys*et al.*, 2006).

Unsaturated (especially polyunsaturated) fatty acids are also more prone to oxidation. In contrast, dietary intake of certain unsaturated fatty acid and fat- soluble antioxidants has been linked to potential health benefit (Gillian *et al.*, 2008). The level of linoleic acid in the oil studied is low (12.84%). Saturated fatty acids with 12, 14 and 16 carbon atoms are known to be the primary contributors of elevated blood cholesterol and so contribute to cardiovascular diseases. Of these, the C_{14} (Myristic acid) is found to be the main culprit (Warldlaw, 2003; Grundy 1999). Hence, the high value (42.743%) of Myristic acid in the oil sample indicates that the oil is not good for human consumption. This observation helps to suggest that *Isoberliniadoka*seed oil has high content of

saturated fatty acids, lauric acid (C12), Myristic acid (C14), Palmitic acid (C16), Stearic acid (C18) and Arachidic acid (C20).

Peak	Component	Fatty acid	Retention time (min.)	Peak area
1	C16:2	Hexadecadienoic acid	0.230	268.498
2	C18	Stearic acid	5.433	6026.6509
3	C12	Lauric acid	9.683	4671.1311
4	C18:2	Linoleic acid	12.846	12862.6949
5	C16	Palmitic acid	23.660	5805.7818
7	C20	Arachidic acid	39.843	4813.3406
8	C14	Myristic acid	42.743	6948.4122

Table4. Composition of fatty acids in the seed oil of Isoberliniadoka

4. CONCLUSION

The results of this study have shown that from nutritional viewpoint, *Isoberlinia Doka* seed oil appears to have a harmful effect on health because of its high content of saturated fatty acids such as: Lauric acid (C12), Myristic acid (C14) and Palmitic acid (C16) which are known to be the primary contributors to elevated blood cholesterol and so contribute to cardiovascular diseases (Grundy, 1999).*Isoberlinia Doka* seed oil could also be considered to be a bad source of oil suitable for food formulation as well as pharmaceutical, soap, paint and perfume industries due to its low level of unsaturation in the fatty acid content compared to other seed oils.

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