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Full Length Research Paper

Physico-chemical and toxicological studies on *Afzelia africana* seed and oil

P. M. Ejikeme[1]*, L. N. Obasi[1] and A. C. C. Egbuonu[2]

[1]Department of Pure and Industrial Chemistry, University of Nigeria, Nsukka, Enugu State, Nigeria.
[2]Nutrition and Toxicological Biochemistry Unit, Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria.

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***Afzelia africana* seeds were obtained from three locations, Abakaliki, Enugu and Nsukka, all in the Eastern part of Nigeria and de-hulled. The seed oil was extracted with (40 - 60°C) petroleum ether and the oil was separated from the solvent using a rotary evaporator. Result of analysis shows that the acid value, saponification value, specific gravity, free fatty acid and refractive index of the oil samples were lowest for the samples obtained from Nsukka. The average moisture content of the seeds from the various locations was 5.56 ± 0.5%. The crude protein (26.44%) and oil content (33.32%) did not vary significantly with location or environment. The various oils were found to contain no less than 0.60 mg/100 g of oxalate, 0.70 mg/kg of phytate and neither tannins or cyanogenic glycosides. This paper empirically highlights the possible industrial applications as well as the safety of the seed oil.**

Key words: *Afzelia Africana* seed oil, phytate, crude protein, average molecular weight, anti-nutritional factors.

INTRODUCTION

Oil seeds used in preparation of diets abound in Nigeria. Seeds of castor, coconut, dikanut, groundnut, melon, oil bean, palm kernel, soybean, *vigna* and *phaseolus* beans cultivars and a wide variety of seeds in the *Leguminosae* family are used in the preparation of diets (Onyeike and Acheru, 2002; Akpanabiatu et al., 2001; Onwuliri and Obu, 2002; Onweluzo et al., 1994). Cereals and legumes in the developing countries supply the energy and vegetable proteins requirement of both humans and animals. Oil seeds serve as the ultimate sources of vegetable oil needs of the world.

Afzelia africana plants are largely cultivated in the Savannah, fringing forest and the drier parts of the forest regions of Africa. The tree is a widespread species with a broad rather open crown and massive branches (most readily recognized by the conspicuous hard blackish fruits), up to 30.5 m high and a girth up to 3 m. It belongs to the family *Leguminosae* and sub-family *caesalpinaceae* (Keay et al., 1964). It is called kawo, apa, akpalata and gayoki by the Hausa, Yoruba, Igbo and Fulani speaking areas of Nigeria, respectively. The seeds have waxy orange cup-like structure at their base and are used in Nigeria generally as soup thickening ingredient in much the same way as melon and irvingia gabonensis seeds. Proximate analysis has shown that the oil seed is a rich source of protein, total carbohydrate and crude fat and potassium (Onweluzo et al., 1995). To the knowledge of the author, there is no literature on the physicochemical properties of the oil from the seeds of the *A. africana* plant. In this paper is reported a preliminary investigation on the physicochemical properties of the fixed oil extracted from *A. africana* seeds. Since climate, soil, nutrition, species, strain and other factors affects the chemical make-up and nutrient value of locally grown food and feeding stuffs (Oyenuga, 1968); it is also the objective of this work to compare the properties of oils extracted from seeds obtained from three different locations. There are numerous toxic constituents of great variety in plants and animals consumed as food. While some plants and animals contain lethal toxins in high concentration and are unfit for consumption, others contain mildly toxic components in low doses and are benign (Macrae, 1993). It is therefore also an objective of this paper to examine some anti-nutritional factors in the

*Corresponding author. E -mail: paul.ejikeme@unn.edu.ng.

Table 1. Chemical Composition of *Afzelia africana* Seed Kernel (g/100 g dry weight sample)*.

Component	Abakaliki	Enugu	Nsukka
Total ash	3.200	3.010	2.860
Moisture	5.876	5.323	5.073
Crude oil	34.576	32.062	33.333
Crude protein (N x5.3)	26.293	26.431	26.596
Carbohydrates (by difference)	30.055	33.173	32.165

*Values average of two determinations.

seed oil.

MATERIALS AND METHODS

Materials

The matured, dried fresh seeds of *A. africana* were bought from the farmers from Enugu and Nsukka as well as Abaliliki in Enugu and Ebonyi States of Nigeria respectively. The seeds were identified at the Department of Botany, University of Nigeria, Nsukka. All the chemicals used are of analytical grades.

Preparation of samples

The seeds were cracked in an aluminium block and immersed in warm water (40 ± 3°C) for 1 h. The water was drained and the swollen husked seeds de-shelled manually with a knife and subsequently dried in an oven at about 50°C for 72h. The dried seeds were crushed and sequentially extracted with petroleum ether (b.p. 40 - 60°C). The oil was separated from the petroleum ether using a rotary evaporator and the oil samples stored in a refrigerator using tight-stoppered brown bottles.

Chemical analysis

Moisture, total ash, crude fiber, oil content and total nitrogen (micro – Kjeldahl) were determined in portions of 5 g of the crushed seeds according to the methods of AOAC (1990).

Physico-chemical characterization of oil samples

The refractive index at 27 ± 0.5°C, using Abbe refractometer as well as the specific gravity also at 27 ± 0.5°C using 10 ml pycnometer, were determined. The acid, saponification and iodine values of the various oil samples were determined, all using ASTM methods (D-1980-67; D-1959-67; D-1962-69) and the free fatty acid calculated. The average molecular weights, MW and the approximate heats of combustion of the oil samples were calculated according to Ibemesi (1992) and Kirk-Othmer (1980), respectively.

The presence or otherwise of hydroxyl fatty acids was quantitatively carried out by the turbidity test (Lakshminarayana, 1967) as described by Nasirulla et al. (1982). 1 ml of filtered, bleached and neutralized oil sample was each dissolved in 10ml petroleum ether (b.p. 40 - 60°C) and acidified with (2%, v/v) concentrated HCl in clean dry test tubes. To this was added down the side of the test tube, without shaking, 1 drop of reagent (1.25 g ammonium molybdate in 100 ml of H_2SO_4, specific gravity = 1.84).

Anti-nutritional factors

1 g each of the oil samples was weighed into a beaker and 15 ml of dilute tetraoxosulphate VI acid added to it. The mixture was boiled in a water bath for 15minutes, cooled and filtered. The characteristic brick - red colouration of glucose was tasted for by the addition of equal volumes of Fehling's solution and boiling for 2 min. Tannins' was determined spectrophotometrically by the methods of Price and Butler, (1977) using Unico. UV-2102 PC Spectrophotometer, Unico. USA. The Thompson and Erdman's procedure (Thomson and Erdman, 1982) was followed for the analysis of phytic acid. Oxalate was analyzed by the methods of Ukpabi and Ejidoh, (1989).

RESULTS AND DISCUSSION

Chemical composition

From the analysis done, the proximate composition of *A. africana* seed kernels, as sampled is presented in Table 1. The total ash and moisture contents were lowest for seeds obtained from Nsukka and highest for the Abakaliki sample. Low moisture content as observed in this work confers good stability (keeping quality) and high yield (Joslyn, 1970; Onyeike et al., 1995; Ijeh et al., 2004; Edem et al., 2009). Values as high as 12.9% has been reported for tropical almond seed (Nwaogu et al., 2008). A minimal variation (5.013 - 5.876) existed between the moisture content of oil samples from various locations; this was reported for soybean oil by Wolf et al. (1982). The average oil content measured by solvent extraction show slight adaptation difference to the various environments; average seasonal temperatures being higher for Abakaliki than for Nsukka for instance and probably giving rise to higher oil content in the former location. A positive correlation has been reported between maximal temperature and oil percentage for soybean (Chapman et al. (1976). The overall high oil content shows that the seed of *A. africana* is a good source of vegetable oil, higher than 19% for soybean (Oyenuga, 1968), 18.53% for almond seed (Nwaogu et al., 2008), 29.39% for custard apple (Amoo et al., 2008) and 23.20% for African pear (Ikhuoria and Maliki, 2007), though lower than melon seed (43%), (Attah and Ibemesi, 1990). Fat- soluble vitamins (A, D, E and K)

Table 2. Physico-chemical characteristics of *Afzelia africana* seed oil*.

Property	Abakaliki	Enugu	Nsukka
Refractive index (27°C)	1.4640	1.4710	1.4650
Specific gravity (27°C)	0.910	0.930	0.890
Acid value (mg KOH/g oil)	3.366	3.142	2.889
Saponification value (mg KOH/g oil)	142.000	128.000	116.000
Iodine value (g I/100 g oil)	114.088	107.870	111.740
Free fatty acid (% as oleic acid)	1.690	1.570	1.440
Average molecular weight	394.980	438.180	483.510
Heat of combustion (kJ/g)	41.723	42.283	42.723

*Values average of two determinations, A= Abakiliki, E= Enugu and N=Nsukka.

dietary fat. From Table 1, the crude protein content of the seeds from Nsukka is higher than that of Enugu and Abakaliki, in that order. The seeds generally have high levels of protein, though the values are slightly lower than that reported elsewhere (Onweluzo et al., 1994) and higher than 29.39% reported by Amoo et al. (2008) and 18.55% by Nwaogu et al. (2008). This high percentage of protein in the seeds is indicative of the fact that it can contribute to the daily protein need of 23.6 g of fats for adults as recommended by National Research Council, (1974). It should be noted that proteins functionally promote growth, tissue repair and maintenance. Dietary proteins are needed for the synthesis of new cells, enzymes, hormones, antibodies and other substances required for the healthy functioning and development of the body as well as its protection (Cheesebrough, 1987). The meal could be a potential source of protein for livestock. The high carbohydrate content of the seed is noted. Nwaogu et al. (2008) though reported a value of 47.35% for *Persea americana* seed.

Physico-chemical characteristics

Results of the physico-chemical characteristic of the oils from the various seed samples are shown in Table 2. The refractive index and the specific gravity of the oil sample, E, were higher than the A and N samples. Specific gravity values of 0.740, 0.9235 and 0.926 were reported for Custard seed (Amoo et al., 2008), *Nicotiana tabacum* L. (Abbas Ali et al., 2008) and tropical almond (Agatemor, 2006), respectively.

The acid values of all the samples are lower than the stipulated permitted maximum values of 10 mg KOH/g and 4 mg KOH/g oil for virgin palm and coconut oils, respectively (Cordex Alimentarious Commision, 1982). The lower the free fatty acid content, the more appealing the oil is (Coenen, 1976). The saponification values were lower than the 188 - 196 for most oils of plant origin (Pearson, 1976). These values are though close to 128.480 mg KOH/g oil obtained by Ikhuoria and Maliki for African pear and by far higher than the 52.11 reported by Amoo et al. (2008).

The iodine values were high, suggesting the preponderance of high molecular weight polyunsaturated fatty acids (Osagie et al., 1986). The iodine value range, 107 - 114 puts the oil in the semi-drying range and it can thus be used in the surface coatings industry to modify alkyd resins. This shows that the oil could be nutritionally beneficial, especially now that vegetable oils rich in polyunsaturated fatty acids and naturally occurring antioxidants are being sourced and recommended to patients that are hyperlipidemic or are suffering from any other lipid disorder (Njoku et al., 2001). Also, the high-unsaturated fatty acid content of the seeds is of importance since they offer protective role against atherosclerotic cardiovascular disease (Odoemena and Onyeneke, 1988).

Calculations show the average molecular weight and heats of combustion, Table 2. The latter were all higher than the value of 39.4 kJ/g ordinarily taken for common edible oils such as lard and cottonseed (Nasirullah et al., 1982). The free fatty acid value in all the oil samples is biologically significant. This is because it has been shown (Bashire, 1971) that in the tropics where vegetable oils are the most common dietary lipids, that it is desirable to ensure that the free fatty acid content of cooking oil lies within the limits of 0.0 - 3.0%.

Anti-nutritional factors

Generally, there is low content of the anti-nutrients reported in this work as shown in Table 3. The supposed glucose extract from the oil did not show a positive result to Fehling's solution, indicating possibly the absence of cyanogenic glycosides in the oil samples. A value of 24.01 mg/100 g of oil was reported for tropical almond (Nwaogu et al. 2008).

Oxalate in large amount binds with calcium to form calcium oxalate, which is insoluble and not absorbed by the body. They are therefore considered poisonous but harmless when present in small amounts as shown in Table 3. The amount of oxalate in *A. africana* seed oil is thus not harmful, more so, when cooking has been reported

Table 3. Some anti-nutritional factors in *Afzelia africana* seed oil*.

Anti-nutritional factor	Abakaliki	Enugu	Nsukka
Cyanogenic glycosides	nd	nd	nd
Oxalates (mg/100 g)	0.43	0.56	0.31
Phytate (mg/kg)	0.38	0.69	0.47
Tannins	nd	nd	nd

*Values average of two determinations; nd: not detectable.

(Eka, 1977; Ekop, 2007) to effect a significant reduction in total oxalate contents of seeds. An unacceptably high value of 27.34 mg/100 g oil was reported for tropical almond (Nwaogu et al., 2008). Like oxalates, phytates chelates di- and trivalent metal ions like zinc, iron, magnesium and calcium to form complex compounds that are not readily absorbed by the intestine, thereby making them unavailable for metabolism (Thompson, 1993). They are easily removed by cooking and soaking (Ihekeronye and Ngoddy, 1985). The highest level of phytates in the oil of Enugu seed sample, 0.69 mg/kg, correlates its highest carbohydrate content and is lower than 1.63 g/ 100 g reported for Kwankiul beans which has been claimed to have no toxic effect on humans. It is lower than 5.44 obtained by Nwaogu et al. (2008) for tropical almond. It has been reported that phytates up to 1% in foods interferes with mineral availability (Anigo et al., 2009).

Conclusions

1. The moisture level of the seeds of *A. africana* is low resulting in low acid value and free fatty acids and consequently high keeping quality of the oil extracted from them.
2. The specific gravities as well as the refractive indices of all the oil samples are within the range reported for other fats.
3. The seeds have high carbohydrate, crude oil and protein contents and are therefore rich sources of energy, dietary lipid and protein.
4. The low acid and saponification as well as the moderately high iodine values of the oil samples suggest that it could help hyperlipidemic patients.
5. It could also be used in the manufacture of soaps and easily digestible margarine, creams and salad oils.
6. The oil is semidrying because of its moderately high iodine value and thus can be used in the manufacture of surface coating agents.
7. The low level of anti-nutrients makes the oil from *A. africana* seeds nutritionally useful.
8. The results of this work and of other works may offer scientific basis for the use of the seeds and the oil may complement current research in the area of alternative sources of industrial vegetable oils.

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