Some Nutrient and Anti-Nutrient Components of *Pterygota macrocarpa* Seed Flour.

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ABSTRACT

The potential of *Pterygota macrocarpa* to serve as a raw material source for industrial applications, domestic consumption, and animal feedstock formulation to reduce the overburden of few seed was assessed by this study. Two seed forms; whole and dehulled seeds, were analyzed for proximate composition, minerals contents, and anti-nutrient properties. Extracted seed-oils were characterized. The results indicate that the seed is a potential source of oil suitable for industrial applications while the mineral components are in favor of its application in feedstock. The seed is a good source of phosphorous, potassium, and zinc. The keep value of the seed is good because of the low moisture content which will ease its preservation. However, domestic application of the seed is limited by low protein content as well as high content values for phytate (approximately 7%) and tannins (1.6 – 2.4%). The study of amino contents, lipid profile, and fatty acid content and development of appropriate processing to reduce the anti-nutrient properties may further reveal the potential domestic application of the seed.

(Keywords: seed analysis, legumes, seed oil, feedstock, domestic use, industrial use)

INTRODUCTION

Thousands of promising species of legumes await research, yet there is overdependence on just a few species because less than twenty out of these thousands are used extensively (Adeparusi, 1998). In many developing countries of the world, development and sustainable growth are serious problems associated with high population growth rates, limited and rapidly diminishing land for food and forage production, etc. These create the need for higher agricultural production and research into full potential of several species of local agricultural crops that abound in such countries but are underutilized.

Pterygota macrocarpa is one of such plants that requires extensive research for potential industrial and domestic utilizations. The plant belongs to the family of mimosaceae. It grows along the lowland rain forest zones of tropical Africa. *P. macrocarpa* is a woody forest product. The plant grows to a large size of about 40m in height and 4m in girth and the seeds are in pod ranging from 8-13cm long to 3-5.5cm wide. When matured and dry, the pod delouses by explosion mechanism with the valves curling outwards to expose the seeds.

The seed is brown in color, smooth in texture and flat shaped. The bark is greenish-grey in color, smooth and used as medicine which is readily available to the vast majority of the rural population in many other developing countries in the world (Keay et al., 1989). It is a legume. The grain legumes include all the cultivated plants or wild species which belong to the family leguminosae (Tabaceae) which is a large family made of 600 genera and 1,300 species (Kochlar, 1978).

The three sub-families of the leguminosae are sometimes classified viz*: mimosaceae, caesalpinaceae and papillionaceae* (Yayock et al., 1995). *P. macrocarpa* belong to the sub-family *mimosaceae*. This work is therefore focused on the evaluation of the proximate composition, minerals, physico-chemical properties of the extracted oil and anti-nutrient contents of the whole and dehulled seed of Pterygota *macrocarpa* with the aim of reporting it potential values for domestic and industrial uses.

MATERIALS AND METHODS

The seeds of *Pterygota macrocarpa*, obtained from Akure, Ondo State, Nigeria, were divided into two parts. One part was dehulled and the other was left as a whole sample. They were thoroughly sun dried, screened to remove undesirable materials such as stones and other impurities. The whole and dehulled seeds were ground into powder, kept in air-tight polythene containers and stored in a refrigerator (4^oC) prior the laboratory analysis.

Proximate Composition of Seed Moisture and Ash Content Determinations: Moisture content of the samples was determined by oven drying at 105^oC while the samples were ashed by placing 5g of the powdered samples in a muffle furnace at 550^oC to a constant weight was obtained with no presence of black particles according to Association of Official Analytical Chemists (AOAC, 1990).

Crude Fat and Crude Fibre Determination: The fat contents of the powdered samples were obtained by weighing 3g of the samples and extracted in continuous extractor of the soxhlet with Petroleum spirit at 60^oC for about 3 hours. Determination of crude fibers was carried out with 3g of the dry powdered samples extracted with light petroleum. The extracted samples were air dried, then treated under standardized condition with boiling dilute H_2SO_4, boiling dilute NaOH, water, dilute HCl, alcohol and ether. The treated samples were then ashed at dull-red heat to determine the crude fiber (Pearson, 1976).

Crude Protein and Carbohydrate Determination: The protein components of the samples were analyzed by using method described by Kjeldahl (Osborne and Voogt, 1978) while carbohydrate content was estimated by the difference of the sum of all the proximate composition from 100%.

Mineral Content Determination in Seed: The mineral compositions of the samples were determined by dry ashing the powder samples in a muffle furnace at 550^oC. The dry ashes were dissolved in 10% HCl and the resulted solutions were used to determine some minerals content of the samples using atomic absorption spectrophotometer-AAS (PYE Unicam sp 9). Flame photometer was used for Na and K while colorimetric method was used for phosphorous determination.

Physicochemical Characterization of the Seed Oil Specific Gravity and Refractive Index Determination: The specific gravity was determined with specific gravity bottle while the refractive index was measured with the aid of a refractometer at 29^0C (Osborne and Voogt, 1978; Pearson, 1976).

Determination of Saponification Value: The saponification value was obtained by adding alcoholic KOH solution to the sample, heated on a boiling water bath. The excess KOH was titrated with 0.5M HCl using phenolphthalein as indicator to assessed the quantity of KOH used up in saponification (AOAC, 1990).

Peroxide and Iodine Value Determination: The peroxide value was evaluated in the dark by dissolving the samples in a mixture of acetic acid and diethyl ether, boiled and poured into a titration flask containing 5% KI. The contents were titrated with 0.002M sodium thiosulphate using starch indicator (Josyln, 1970). Iodine value was estimated by method described by Joslyn (1970).

Acid Value and Free Fatty Acid Determination: Acid value and free fatty acid were determined by dissolving the samples in a percentage (v/v) of alcohol and then titrated with 0.1M KOH using phenolphthalein indicator (AOAC, 1990).

Determination of Phytate, Tannins and Oxalate: The phytate contents of the samples were determined by using the method of Young and Graves (1940) while the method described by Markkar and Goodchild (1996) was used to evaluate the tannins content of the samples. Oxalate was analysed by treating the powdered samples with 0.75 M, H_2S0_4 stirred and filtered using Whatman No 1 filter paper. The filtrates were then titrated hot (80 - 90 ^oC.) against standard potassium permanganate to a persistent faint pink color (Day and Underwood, 1986).

STATISTIC ANALYSIS

All the experiments were carried out in triplicates and the mean ± standard deviation are reported.

Data were subjected to analysis of variance [ANOVA]. Significance of means differences were determined (Duncan). Significance was accepted at $P < 0.05$.

RESULTS

<u>Proximate Composition</u>: The proximate composition of the whole and dehulled seed flour samples is presented in Table 1. From the table mean values of ash content, moisture content, crude fiber and carbohydrate are higher for the whole seed than the dehulled. However, the crude protein and the crude fat contents of the dehulled seed sample are significantly higher than the whole seed. The most significant change in composition between the seed samples is witnessed in the carbohydrate content. All parameters investigated are significantly different from each other in both seed types. The major component of the seed is crude fat followed by crude protein and crude fiber. The seeds have very low carbohydrate content particularly the dehulled seed. The high dry matter is desirable for preservation purpose.

<u>Mineral Contents</u>: Table 2 presents the mineral contents of the whole and dehulled seed samples. The concentrations of the minerals are presented in percentage (%). Phosphorus and potassium are the major mineral contents of the samples while Pb and Mn are not detected in the sample. The mineral components in the dehulled are more than the whole seed and there are significant differences in these components in the two seed forms except for Zn which is not significantly different in the two seed forms studied. The seed was observed to exhibit higher concentration of Zn, being a micronutrient, than even Ca, Mg and Na which are macronutrient elements. Ca is the least mineral among those studied and detected in the seeds.

<u>Physicochemical Characterization of Seed Oil Extracted</u>: The largest component of the seed is crude fat and oil. This was further studied to understand its composition for potential applications. The results of the physicochemical characterization of the oil extracts are presented in Table 3. All the parameters considered in the dehulled seed-oil are lower in value than in the whole seed sample. However, the specific gravity and the refractive index of the two seed oils are not significantly different for each other as against the other parameters which differ significantly (at $p<0.05$) from each other. The change observed in the saponification value and the iodine value between the whole seed and the dehulled seed are very significant and notable. There is about 50% drop in these values from the whole seed extracted oil to the dehulled seed one.

<u>Anti-nutrient Components</u>: The final set of components investigated in the seed is the anti-nutrient components. The results of these components in the different seed forms are presented in Table 4. The presence of these toxic substances otherwise known as anti-nutritional factors is one of the main draw backs limiting legumes (Aiyesanmi and Oguntokun, 1996). The results show that phytate level (mg/g) in the whole seed sample is higher than that of the dehulled sample. The levels of phytate in these samples seemed to be much lower than that reported for *Dioclea reflex* seed (Aiyesanmi and Oguntokun, 1996). The low levels of phytate in the studies samples may not therefore seriously affect some of the samples nutrients. The tannins (%) present in the samples are 7.00 ± 0.04 for the whole seed and it is slightly lower than that of the dehulled seed (7.33 ± 0.02). The two values are significantly difference ($P<0.05$). These values are higher than 1.52% reported *D. reflex* seeds (Aiyesanmi and Oguntokun, 1996).

DISCUSSION

The proximate composition (%) presented in Table 1 shows that the ash content of the seed, both whole and dehulled is relatively low when compared with other legumes like African yam bean with ash content 3.87% (Oshodi et al., 1997). Ash content in food is an indicator of the inorganic mineral content and the quality of the food sample. The mineral may be toxic or essential. Though, the mineral components of this seed are relatively small depicted by the low ash content, they are non-toxic but essential mineral for metabolic activities in living tissues (Table 2). The mineral contents of the seed improve significantly with dehulling. The concentrations of these minerals present in the seeds make it a good source of phosphorus and potassium as macronutrients and zinc as micronutrient. Zinc is an antioxidant and is needed for some hormone and enzyme formation. Its presence in high quantity is a positive factor to the potential usage of the plant. Phosphorous content in the seed is desirable for energy storage and transfer; cell division and reproduction among others.

Table 1: Proximate Composition (%) of the Samples.

Proximate Composition (%)	WHOLE SEED	DEHULLED SEED
Ash	$2.30^{b} \pm 0.01$	$1.57^{a} \pm 0.02$
Moisture	$7.27^{c} \pm 0.03$	$3.67^{a} \pm 0.04$
Dry matter	$92.73^{c} \pm 0.03$	$96.33^{e} \pm 0.04$
Crude protein	$7.21^{a} \pm 0.12$	$10.81^{c} \pm 0.28$
Crude fat	$63.97^{c} \pm 0.23$	$74.40^{e} \pm 0.57$
Crude fibre	$9.41^{d} \pm 0.05$	$7.58^{c} \pm 0.02$
Carbohydrate	$9.84^{e} \pm 0.21$	$1.97^{b} \pm 0.42$

Values with different superscripts in each row are significantly difference (P<0.05)

Table 2: Mineral Content of the Samples.

Mineral Content (g/100g)	WHOLE SEED	DEHULLED SEED
Calcium	$5.81^{a} \pm 0.03$	$6.50^{b} \pm 0.05$
Magnesium	$22.50^{b} \pm 0.03$	$24.06^{c} \pm 0.03$
Sodium	$14.95^{a} \pm 0.02$	$25.88^{d} \pm 0.06$
Potassium	$213.03^{d} \pm 0.16$	$324.15^{c} \pm 0.46$
Zinc	$26.23^{c} \pm 0.06$	$28.06^{c} \pm 0.04$
Phosphorous	$286.89^{e} \pm 0.26$	$475.96^{f} \pm 0.45$
Manganese	ND	ND
Lead	ND	ND

ND = Not detected, Values with different superscripts in each row are significantly difference (P<0.05)

Table 3: Physico-Chemical Properties of the Extracted Oil of the Samples.

Physicochemical Properties	WHOLE SEED	DEHULLED SEED
Specific gravity at 20^{0}C	$0.930^{f} \pm 0.00$	$0.928^{d} \pm 0.00$
Refractive index at 29^{0}C	$1.493^{cb} \pm 0.04$	$1.455^{b} \pm 0.06$
Acid value (%)	$4.12^{e} \pm 0.00$	$3.98^{f} \pm 0.38$
Iodine value (%)	$93.31^{f} \pm 0.13$	$49.68^{d} \pm 0.15$
Peroxide value (mg-KOH/g)	$6.07^{d} \pm 0.12$	$4.87^{c} \pm 0.12$
Saponification value (mg-KOH/g)	$43.85^{c} \pm 0.95$	$17.88^{b} \pm 0.11$
Unsaponification value (%)	$1.23^{d} \pm 0.01$	$1.02^{c} \pm 0.02$
Free fatty acid (as oleic acid)	$2.16^{f} \pm 0.02$	$2.05^{d} \pm 0.02$

Values with different superscripts in each row are significantly difference (P<0.05)

Table 4: Anti-Nutrient Composition of the Samples.

Anti-Nutrients	WHOLE SEED	DEHULLED SEED
Tannins (%)	$7.00^{d} \pm 0.04$	$7.33^{e} \pm 0.02$
Phytate (%)	$2.40^{d} \pm 0.08$	$1.57^{c} \pm 0.05$
Oxalate (%)	$0.4^{b} \pm 0.01$	$0.55^{e} \pm 0.01$

Values with different superscripts in each row are significantly difference (P<0.05)

The low concentration of Na is equally desirable since high Na is a risk factor for hypertension but low Ca is a disadvantage, since Ca is needed for bone formation; muscle contraction; nerves activities among others. Generally, the non detection of toxic metals in the seed sample makes it a safe source for domestic consumption and feedstock formulation. It is also an indication of reduced environmental effects on the plant from the sourced location.

Furthermore, the values obtained for the moisture content and the associated dry matter of the seeds are suitable for an increase shelf-life of the seed and seed-products. High moisture content aid microbial growth and reduce shelf-life of food products. Thus, the reduced moisture content of the seed especially the significant drop in the moisture content after dehulling serve as a positive processing step that will improve the quality of the seed product. This also will reduce the cost of preservation and processing of the seed for both industrial and domestic uses. The crude protein content of the seed however, limits its potential usage for food and feed formulation because of its low content unlike others with higher contents (Amoo, 1998).

The dehulling also improved the crude protein contents significantly. An evaluation of the amino acid contents will reveal better the value of the protein in the seed. Presence of essential amino acids in the seed will enhance its value despite the low crude protein content. The crude fibre content of the seed is equally a good and useful part of it that is essential for colon digestion in humans and the percentage obtained is suitable for this purpose but the seed is not a good source of carbohydrate.

The highest content of the seed is crude fat and oil which amount to 64% of the whole seed and 74% of the dehulled seed indicating improvement in quantity by dehulling. The seed is therefore a veritable source of fat and oil with both potential domestic and industrial usages. The nutritive value of the fat and oil for possible domestic exploration is better expressed in it being the most compact energy source available; an important nutrient for body metabolism; heat regulation and solvent for fat soluble vitamins. Therefore, the physicochemical characterization of the seed-oil extract was carried out and the results show that the oil is stable with low acid value (AV), peroxide value (PV) and free fatty acid (FFA) which are indication of slow rate of deterioration of the oil; stable shelf-life and suitability for domestic consumption (Esuoso and Odetokun, 1995).

The unsaponifiable matter in the seed oil is also low and may be an indication of low values of natural products like steroids, alkaloid and others which can limit the domestic consumption of the oil by toxicity increase. A further assessment of the fatty acid content and the lipid profile of the seed oil will confirm further the domestic consumption value of the seed-oil. However, the values obtain for the iodine value classified the oil as semi-drying oil which is suitable for use industrially in the production of alkyld resin due to the unsaturation in the oil. The saponification values are also favorable for recommendation of the oil as suitable blend in soap-making and for transesterification for biodiesel production.

Finally, the potential usage of the seed for human consumption and animal feed formulation is limited by the values obtained for the anti-nutrient properties (Table 4). Reactions of anti-nutrient in seed with proteins and mineral components have been documented in literature (Siegenberg et al., 1991). Phytate which represent about 89% of the total phosphorous concentration is stored in cereal grains and other seed and is known to interact with both iron and proteins and lowers the bioavailability of minerals and inhibits several proteolytic enzymes and amylases (Siegenberg et al., 1991).

Existence of variability for phytate is less known; however, heat-stable phytate is believed to reduce bioavailability of nutrients more since its phosphate groups can readily complex with cations such as iron, zinc and calcium and with protein. Moreover, with the high values of tannins in the analyzed samples, the samples are expected to have poor nutritive values. Tannins have been long reported as anti-nutrients, both for their negative effect on protein digestibility (Bressani et al., 1982) and on mineral nutrition (Rao and Prabhavathi, 1982; Siegenberg et al., 1991). Most tannins in legume seeds are found in the seed coat but a variation was observed in this study with the dehulled seed having higher tannin concentration. The oxalate values falls within the range (1.7 – 6.5) mg/g reported for some oil seeds (Victor and Olubunmi, 2003). Generally, processing effects such as shredding, roasting, blanching, sun drying among others have been suggested to reduce or eliminate some of the anti-nutritional factors inherent in plant seeds and leaves (Santish and Chauha, 1986; Fagbemi, 1999; Amoo, 2004; Fasuyi, 2005). This will improve their nutritive values and enhance their domestic applications.

CONCLUSION

Pterygota macrocarpa is a potential seed for industrial and domestic usages. It is a good source of fat and oil raw material for various industrial uses. The high fat contents of both the whole seed and the dehulled seed make the sample a good source of fat for consumption in addition to any industrial purposes. Industrially, the whole seed oil is suitable for soap making and biodiesel production due to its high saponification value. As a semi-drying oil, with high iodine value, the whole and dehulled seeds indicate suitable unsaturation required for alkyld resin synthesis. The high contents of some of the valuable minerals will go a long way to solve the mineral related problems in the consumers. However, the potential uses of this seed are limited by low protein content and high concentration of anti-nutrient compounds. High tannin and phytate contents of the analysed samples may not make the samples protein bioavailable. Further studies on the amino acid contents, fatty acid contents and the use of food processing to reduce anti-nutrient components' concentration may reveal further the usefulness of this seed.

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