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PHYSICAL PROPERTIES OF SEEDS OF AFRICAN WALNUT (*PLUKENETIA CONOPHORUM* MUELL ARG.) FROM SOUTHEASTERN NIGERIA, AND THE EFFECT OF BOILING ON THE PROXIMATE QUALITIES

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ABSTRACT
A study of the physical and proximate traits of seeds of African walnut (Plukenetia conophorum) from four locations (states) in south-eastern Nigeria was carried out in 2011. The locations considered were Enugu, Anambra, Abia and Rivers. Results of statistical analysis showed remarkable variation ($p < 0.05$) in all the seed physical traits across the sampled locations. Accessions from Anambra were outstanding in seed weight, seed circumference and seed diameter. However, the Enugu accession was the best in terms of seed edible proportion, followed by that of Abia, while seeds obtained from Anambra had the least percentage edible proportion. The accession from Rivers had the least seed weight, kernel weight, seed circumference and seed radius. Correlation coefficients among the physical traits indicated that seed weight had significant statistical linkage with all the other physical traits except seed coat thickness. Seed coat thickness, just like the seed edible proportion had poor statistical linkage with most of the seed physical traits; whereas kernel weight recorded a high significant positive relationship ($r = 0.913^{**}$) with seed weight. Kernel weight, seed volume, seed circumference and seed diameter showed more or less similar pattern of relationship. Principal Components Analysis (PCA) results identified five physical traits including seed weight, kernel weight, seed volume, seed circumference and seed diameter as the most discriminatory traits among the accessions. Location significantly ($p < 0.05$) influenced only moisture, fat and ash content of the seeds. Fat concentration of the Anambra and Abia accessions were higher than those of the other two accessions. Boiling lowered the moisture content of seeds and significantly ($p < 0.05$) increased the fibre content. Location × processing interaction had no remarkable effect on the proximate attributes of the seeds. Correlation analysis linked high fat content with large kernel diameter. Thus, seeds with large kernels could be selected for fat content and those with small size could give higher percentage edible proportion. Seeds of African walnut in the sampled zone have adequate nutritional content to qualify as a protein-rich oilseed and have shown enough variability to warrant selection for further improvement.

Keywords: Physical and proximate traits, African walnut, boiling treatment, southeast Nigeria.

INTRODUCTION
African walnut (*Plukenetia conophorum* Muell Arg**.**) is a perennial climbing species belonging to the family Euphorbiaceae (Amaeze *et al*., 2011). The species is found in southern Nigeria and produces seeds from June to September (Egarhevba *et al*., 2005). *Plukenetia conophorum* is better appreciated for its seeds which are extensively consumed by people inhabiting the southern region of Nigeria, where the species is common (Akpuaka and Nwankor, 2000). The importance of the seeds is quite pronounced given that the glut harvest period (from June to September) coincides with a low availability of other fruits. Local trading in the seeds of African walnut has been reported to improve the incomes of rural dwellers (Egarhevba *et al*., 2005). Nutritional importance of the seeds of African walnut has been supported by scientific research. Proximate analysis of the seeds by Enujiugha (2003) showed protein and fat content of

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29.09% and 48.90%, respectively (on dry weight basis).

The usefulness of African walnut goes beyond the consumption of its seeds as snacks. Almost every part of the plant appears to have significance in traditional medicine. Ajaiyeoba and Fadare (2006) have reported on the potency of the nuts and leaves in addressing male infertility problems and dysentery, respectively. The macerated leaves and roots are used traditionally in the treatment of asthma and hypertension (Okafor and Okorie, 1990). A recent study by Ayoola *et al*. (2011) showed the rich phytochemical, vitamin and mineral content of the root of *Plukenetia conophorum* which explains the usefulness in traditional medicine and the potential in the formulation of new drugs.

Assessing the physical attributes of fruits, seeds or kernels of a species over a given geographical area could serve two important purposes; first, is the design and fabrication of appropriate engineering equipment for the handling and processing (Dash *et al*., 2008; Galedar *et al*., 2008). Second, is to aid selection for the genetic improvement of the species. In *Plukenetia conophorum,* the two options are more or less equally important owing to the preponderance of basic nutrients in the seeds. For instance, the oil content of the seeds (48.9%) is high enough for commercial exploitation (Enujiugha, 2003). At the moment, the species is yet to be fully domesticated although the need for this has been emphasized (Egarhevba *et al*., 2005; Ayoola *et al*., 2011).

For the purpose of selection, provenance evaluation is necessary. In this species however, we have not been able to cite any report assessing either physical or chemical attributes of seeds across locations. Such reports where available have been based on samples collected from a single location. This paper which reports on the physical and proximate attributes of the seeds of African walnut from the south-eastern zone of Nigeria represents an effort towards accelerated domestication of the species to foster a better and more sustained exploitation of its potentials.

MATERIALS AND METHODS

Seeds of *Plukenetia conophorum* were collected from four states in south-eastern Nigeria in 2011. The four states were Enugu, Anambra, Abia and Rivers. In determining the seed physical traits, a representative sample of 100 seeds were collected from each location and divided into groups of ten seeds, with each group considered as a replicate. Physical properties measured were seed weight, pulp weight and volume, pulp diameter, seed volume, seed circumference, seed radius and seed coat thickness. The seed edible proportion was calculated as the ratio of the pulp weight to whole-seed weight multiplied by 100%.

Proximate properties of the accessions were determined in triplicates using raw and boiled seeds across the four locations, following the procedures outlined by the Association of Official Analytical Chemists (AOAC, 1980). A 2 g sample of the milled seed pulp was extracted with petroleum ether in a soxhlet apparatus and the extracted fat was oven dried at 100 °C after which it was weighed and percentage fat content estimated. Crude protein was estimated by the micro-Kjedahl method which multiplies estimated Nitrogen content by a factor of 6.25 to obtain crude protein. Ash content was determined by incinerating a 2 g of sample in a muffle furnace at 600 °C until ash was obtained. The Weende method which involved hydrolysing the protein, fat, starch and other digestible carbohydrates out of the sample was employed for estimating fibre content. Carbohydrate content was obtained by subtracting the values of other proximate traits from 100%.

Data collected were subjected to analysis of variance (ANOVA); mean separation, where applicable, was carried out using F-LSD at 5% probability level. Principal component analysis (PCA) was used to identify traits capable of being used for African walnut seed classification in Nigeria. The statistical software used for the analyses (ANOVA and PCA) was GENSTAT Discovery Edition 3, Release 7.2DE (GENSTAT, 2007). In addition, correlations among traits were assessed by the use of SPSS Release 17.0 (2008).

RESULTS

Physical Traits: All the seed physical traits of *P. conophorum* studied varied significantly ($P<0.05$) across the sampled locations (Table 1). Seeds from Anambra had significantly higher values of seed weight, seed circumference and seed diameter. Its seed radius was similar to that of the Enugu accession. Anambra accession also showed superior performance in some kernel characters like kernel weight and kernel diameter. However, in terms of edible proportion (kernel) of seed, the Enugu accession was outstanding (74.1%), followed by that of Abia (70.7%). Seeds obtained from Anambra had the least percentage edible proportion (64.8%). The accession from Rivers had the least seed weight, kernel weight, seed circumference and seed radius.

Correlation coefficients among the physical traits as summarised in Table 2 indicates that seed weight had significant statistical linkage with all the other physical traits except seed coat thickness. In particular its correlation with the seed edible proportion was low and negative but significant ($r = -0.372^*$). Kernel weight, seed volume, seed circumference and seed diameter showed similar pattern of relationship except that their correlation with seed edible proportion, even though negative, was not significantly so. Seed coat thickness had very low negative relationships with all the seed physical traits thus, did not register any significant correlative response. Similarly, the seed edible proportion had poor statistical linkage with most of the other seed physical traits, whereas kernel weight recorded a high positive significant relationship (r = 0.913**) with the seed weight. Results of Principal Components Analysis (PCA) (Table 3) showed an explanation of about 84% of the total variation by the three principal axes retained, with the first component axis accounting for more than half of the total variability. Kernel weight and seed weight had the highest eigen vector values on PRIN 1, while seed edible proportion and kernel diameter had the highest scores on PRIN 2. On the third principal axis (PRIN 3), seed edible proportion still recorded the highest eigen vector score. Going by the latent roots, five physical traits including seed weight, kernel weight, seed volume, seed circumference and seed diameter accounted for 56.21% of the total variability existing among the seeds of African walnut from the four states of southeastern Nigeria. The seed edible proportion and kernel diameter accounted for 15.40% of the total variation, while edible proportion recorded an additional 12.34% of the total variability. These results suggests that seed weight, kernel weight, seed volume, seed circumference, seed diameter, seed edible proportion and kernel diameter accounted for about 84% of the total variability recorded among the seeds evaluated.

Table 1: Physical traits of seeds of African walnut from selected locations in south-eastern Nigeria

Location	Seed weight (g)	Seed edible proportion (%)	Kernel weight (g)	Seed volume (cm^3)	Kernel diameter (cm)	Seed circumference (cm)	Seed coat thickness (mm)	Seed diameter (cm)	Seed radius (cm)
Enugu	7.7	74.1	5.9	6.4	1.9	9.5	1.03	3.0	1.52
Anambra	11.3	64.8	7.5	7.7	2.2	9.7	1.06	3.1	1.55
Abia	8.5	70.7	6.1	6.6	2.1	9.2	1.22	2.9	1.47
Rivers	7.2	66.7	4.9	4.7	2.0	9.0	1.18	2.9	1.46
$LSD_{(0.05)}$	0.52	3.09	0.46	0.85	0.07	0.21	0.140	0.06	0.04

Table 2: Correlation coefficients among physical traits of seeds of African walnut from south-eastern Nigeria

	Seed radius (cm)	Seed diameter (cm)	Seed coat thickness (mm)	Seed circum. (cm)	Kernel diameter (cm)	Seed volume (cm^3)	Kernel weight (g)	Edible portion (%)	Seed weight (g)
Seed weight	0.562*	0.652*	-0.146	0.690*	0.779	0.729*	0.913	-0.372	
Edible proportion	-0.066	-0.122	-0.147	-0.119	-0.248	0.013	-0.060	-	
Kernel weight	0.531**	0.667**	-0.184	0.711**	0.797**	0.828**	-		
Seed volume	0.440**	0.566**	-0.249	0.625**	0.663**	-			
Kernel diameter	0.255	0.361*	0.042	0.413**	-				
Seed circumference	0.821**	0.978**	-0.191	-					
Seed coat thickness	-0.148	-0.191	-						
Seed diameter	0.840**	-							
Seed radius	-								

Table 3: Eigen vector values of physical traits of seeds of African walnut from south-eastern Nigeria

Physical traits	**PRIN 1**	**PRIN 2**	**PRIN 3**
Seed weight (g)	**-0.40545**	0.24605	-0.04224
Edible proportion (%)	0.0865	**-0.50929**	**-0.51471**
Kernel weight (g)	**-0.41009**	0.12047	-0.25694
Seed volume (cm^3)	**-0.36485**	0.04155	-0.36907
Kernel diameter (cm)	-0.31765	**0.46295**	-0.27296
Seed circumference (cm)	**-0.39647**	-0.23916	0.25239
Seed coat thickness (cm)	0.09937	0.45796	0.40514
Seed diameter (cm)	**-0.38381**	-0.27812	0.29984
Seed radius (cm)	-0.33401	-0.32443	0.37257
Latent roots	5.059	1.386	1.111
% of variation explained	56.21	15.40	12.34

Table 4: Influence of location and processing on proximate qualities (%) of seeds of African walnut from selected locations in south-eastern Nigeria

Treatment	Moisture	Carbohydrate	Protein	Fat	Fibre	Ash
Location						
Enugu	8.8	35.8	23.5	21.0	5.0	5.8
Anambra	8.8	32.9	21.4	25.9	5.2	5.8
Abia	7.3	34.0	23.6	25.0	4.5	5.0
Rivers	8.0	38.6	20.8	22.2	5.2	5.1
LSD (0.05)	0.77	NS	NS	2.63	NS	0.65
Processing						
Boiled	7.8	34.1	22.2	24.0	6.2	5.7
Fresh	8.6	36.6	22.4	23.0	3.8	5.2
LSD (0.05)	0.54	NS	NS	NS	0.85	NS

Table 5: Correlation coefficients between physical and proximate traits of seeds of African walnut from south-eastern Nigeria

Seed traits	Moisture	Carbohydrate	Protein	Fat	Fibre	Ash
Seed weight	0.363	-0.836	-0.196	0.821	0.175	0.491
Edible proportion	-0.060	-0.091	0.869	-0.602	-0.520	0.067
Kernel weight	0.413	-0.928	0.065	0.732	0.038	0.601
Seed volume	0.352	-0.971*	0.308	0.657	-0.151	0.590
Kernel diameter	-0.125	-0.690	-0.315	0.980*	-0.039	-0.030
Seed circumference	0.749	-0.743	0.146	0.323	0.243	0.900
Seed coat thickness	-0.966*	0.243	-0.036	0.241	-0.547	-0.987*
Seed diameter	0.831	-0.612	-0.164	0.336	0.501	0.901
Seed radius	0.837	-0.641	0.000	0.262	0.404	0.939

*Correlation is significant at the 0.05 level of probability.

Proximate Composition: Place of sample collection (Location) significantly influenced only moisture, fat and ash content of the seeds (Table 4). Seeds from Abia had the least moisture and ash content, although with the latter trait, they did not differ statistically from those of Rivers. Fat concentration of the Anambra and Abia accessions were higher ($P<0.05$) than those of the other two accessions. Similarly, processing of the seeds by boiling did not show much significant response. Moisture content of boiled seeds was lower than that of raw seeds. Conversely, boiling increased the fibre content of the seeds. Interactive effect of location and processing had no remarkable effect on the proximate attributes of seeds of African walnut (Data not shown).

Correlation coefficients between proximate traits and physical traits of seeds of African walnut as presented in Table 5, showed high negative significant correlation (r = -0.971*) between seed volume and the carbohydrate content. The relationship between kernel diameter and fat content was highly positive and significant (r = 0.980*). Seed coat thickness was negatively but significantly correlated with moisture (r = -0.966*) and ash content (r = -0.987*), respectively. Carbohydrate had a negative relationship with most of the seed physical traits. Protein content recorded a high positive but non-significant relationship (r = 0.869) with the seed edible proportion. Similarly, high positive but non-significant relationship exists between ash content and seed circumference (r = 0.900), seed diameter (r = 0.901) and seed radius (r = 0.939), respectively.

DISCUSSION

The variation in physical traits of seeds of African walnut across the sampled locations in south-eastern Nigeria could be due to such factors as climatic and soil conditions of the locations, genetic constitution and human selection. In *P. conophorum*, a planned investigation into the level of human intervention has not been done. However, with the level of utility achieved by the species, it is proper to assume a reasonable level of human selection which could account for some of the discernable variations across the accessions. Variation in physical traits of indigenous species across sites is a common phenomenon and has been reported for shea *Vitellaria paradoxa* (Maranz and Wiesman, 2003; Ugese *et al.*, 2010a), *Dacryodes edulis* and *Irvingia gabonensis* (Leakey et al., 2004), *Balanites aegyptiaca* (Abasse *et al.*, 2010) and a host of other species.

The importance of seed weight has been demonstrated by its significant statistical linkage with all other physical traits. Its negative significant linkage with seed edible proportion is an indication that heavier seeds may have a high proportion of seed coat which is inedible, hence the observed negative correlation. Thus, selection for edible proportion would favour smaller seeds over the bigger ones.

Moreover, seed coat thickness was negatively correlated with the moisture and ash contents of the edible pulp. This is understandable, since thicker coat would restrict moisture imbibition by the seeds even during boiling. More so, the would-be available minerals were allotted to the non-edible seed coat. This suggests possible utilization of the seed coat in livestock feed, nursery media formulation and as soil amendment.

Principal components analysis showed that seed size indicators (including seed weight, kernel weight, seed volume, seed circumference, seed diameter, seed edible proportion and kernel diameter) were the most discriminating traits in the classification of seeds of African walnut in south-eastern Nigeria. These traits are important for selection purposes and have also shown interesting correlations among themselves, which is capable of making the task of selection even much easier.

Proximate qualities of moisture, fat and ash were influenced by location such that Anambra and Abia accessions had higher values compared to the other locations. Variation in proximate traits has been reported for fruits and seeds of the shea tree and has been largely ascribed to differences in climatic conditions (Ugese *et al*., 2008; 2010a&b). In the present study, variations in climatic variables within the study area may not be as wide as those of the savanna region where the shea tree was reported. As such, differences in soil fertility and/or some genetic factors may play a more crucial role in determining variability in seed size and proximate traits of African walnut in the study area.

Effect of boiling appeared to have impacted favourably on proximate qualities of African walnut as it reduced moisture content and increased proportion of fibre in seeds. Important health benefits of dietary fibre include ease of digestion, bowel movement and reduced rate of cancer (ENCARTA, 2009). The traditional practice of boiling seeds of African walnut before consumption (Akpuaka and Nwankor, 2000) is a good one and should be continued. This is further reinforced by the reported reduction of anti-nutritional factors due to boiling (Enujiugha, 2003).

Although the numbers of significant correlations between proximate and physical attributes of seeds of African walnut were few, they nevertheless showed interesting relationships with some implications for germplasm selection. In particular, the positive significant statistical linkage between kernel diameter and fat content could be used in selecting for seeds with high fat content. This is plausible considering the high oil content of seeds of African walnut which makes it qualify as an oilseed. In this respect, seed weight and kernel weight could also be used as indices for high oil content; the relationship between the two traits and fat content is positive and reasonably high. Also, since kernel diameter and seed weight showed positive significant inter-relationship, it is logical to infer that any of the seed size indicators could be used in selecting seeds for high fat content.

It seems clear that seeds with high fat content would not also have high edible portion as shown by the nature of relationship between the fat content and percentage edible proportion. Thus, as progress is made in the utilization and domestication of this species, selection would proceed along two lines: large seeds for the oil seed market or crop and smaller seeds for the edible seed market. In any case, size is an important criterion in any fruit market as larger seeds would always command higher premium.

CONCLUSIONS

1. Physical traits of seeds of African walnut show remarkable variability in south-eastern Nigeria.
2. Location of seed collection significantly influenced proximate attributes of moisture, fat and ash while boiling reduced the moisture content of seeds but increased their proportion of fibre.
3. Bigger seeds tended to be linked with high oil content while smaller ones are associated with higher percentage edible proportion.

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