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Morpho-physical and Nutritional Characterization of Seeds and Tubers of Sphenostylis stenocarpa (hochst ex a. Rich.) Harms

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ABSTRACT

Comparative study of the morpho-physical characteristics of two varieties of seeds of Sphenostylis stenocarpa and the evaluation of the nutritional potential of seeds and tubers revealed significant differences in seed density and color. Black seeds are less bulky and denser (1.5 g/mm³) than brown seeds (1.2 g/mm³). Seeds are richer in protein (23% and 27%) and fat (2%) than tubers (8-10 and 0.8-0.5%) while the last-mentioned are richer in starch (85%). Splitting of the fibers shows that the seeds are rich in soluble fibers at a high molecular weight while the tubers are rich in insoluble fibers. Sequential splitting of proteins reveals that salino-soluble and glutelin are the predominant protein groups of seeds and tubers of Sphenostylis stenocarpa. In regard to the salino-soluble, globulins are in the majority in seeds (38-41%) contrary to tubers (3%), and the last-mentioned are richer in albumins (44-56%) than seeds (1, 2-8%). Glutelin G2 and G3 are more concentrated in seeds (30-37%) than tubers (12%). Prolamins are present only in trace form in both organs..

Keywords: *Sphenostylis stenocarpa*, Nutritional potential, Sequential splitting

INTRODUCTION

Africa in general and the West Africa in particular, have made remarkable efforts in regard to the population suffering from hunger. This has enabled it to achieve target (1 c) of Millennium Objective for the Development by reducing the hungry population of 60% [1]. The population suffering from malnutrition is reduced approximately to 11 million between 1990-1992 and 2014-2016. Nevertheless, this progress does not allow to achieve the objective aimed by the World Food Summit (WFS) which consists to reduce by half the number of undernourished people in 2015 (FAO, 2015).

In a context where alone Benin have, out of medicinal species, spices and oil-bearing plants, of more of 38 neglected and under-exploited plant species used for human consumption [2] and considering the role of Agriculture in food security, local agricultural products should be upgraded. This upgrading requires deep scientific research with a view to access a good knowledge of neglected and under-exploited agricultural resources.

The results of this research would constitute concrete tools to sensitize the population in order to harden them to pass over the taboos of these products and promote their popularization. The nutritional potential of these resources would be put at the service of food security and an increase in agricultural incomes will result.

Among the great diversity that characterizes agricultural production in African rural areas, appears the African Yam Bean (*Sphenostylis stenocarpa*). The last-mentioned is a tuberous legume belonging to the resilient, neglected vegetable species and therefore under-exploited. These seeds are known to be rich in protein (21%) and carbohydrate (24-68%) [3,4] while tubers are richer in protein than sweet potatoes, Cassava and potato [5]. It is a plant resistant to environmental factors [6], creeping so it is cultivated very easily in association with others plants and finally, fixes the nitrogen of the soil, and therefore does not require fertilizers. Morphologically, it is the most variable species of its kind and the most economically profitable [6]. Thus, we observe the varieties with black seeds, brown seeds, whitish

or spotted seeds [7]. This diversity of varieties is often subjected to controversy considering the significant differences characteristic of one variety to another.

Several authors have carried out research on the agronomic and genetic aspects of the species and also on the nutritional potential of the seeds. Very little researches are interested at tubers which constitute a resource as important as seeds especially in Zaire/Congo where leaves are consumed as vegetables and tubers are cooked and eaten [6]. This study aims to determine the morpho-physical characteristics of the seeds and nutritional characteristics of the seeds and tubers of *Sphenostylis stenocarpa*.

MATERIALS AND METHODS

Origin of the studied samples

The samples of *Sphenostylis stenocarpa* used in this study are some black and brown seeds. They are two varieties of species harvested in the Democratic Republic of Congo in the province of Bandundu, specifically in the town of Ngidinga (black seeds) and of Feshi (brown seeds). Mature and dried seeds were manually extracted from the cloves.

The tubers chips are from Feshi variety. Harvested at maturity, tubers have been peeled (for white flour), washed and dried under sun, and ground with mill hammer.

Determination of seeds size

The size of the seed varieties are measured following their length (L), width (l) and thickness (e) by a digital display (Mitutoyo Corporation, Japan) on 30 seeds taken randomly from the amount of each variety. The measurement has been performed with a precision of 0.05 mm. The volume (V) of each seed was calculated by considering an ellipsoidal morphology according to the formula:

$V=4/3\pi\ (L \times l \times e)$

The mean volume of a seed and a standard deviation were calculated by considering the individual values of the 30 seeds.

Determination of the density and weight of 1000 seeds

Weight of 1000 seeds (M) was obtained from the measured weight of three batches of 200 seeds, selected at random from the seed population of each variety. The density (ρ) of the seeds was calculated by considering the average of a volume of one seed (V) and the weight of 1000 seeds according to the formula:

$P=M/(1000 \times V)$

Determination of sample color

The parameters of color of the whole seeds were determined using a spectro-colorimeter (Hunterlab Miniscan XE Restor VA 3.5), calibrated with a referenced standard plate (white). The measurement was carried out on whole seeds placed in a cup with a white background that is opaque, and a diameter corresponding to the scales of the spectro-colorimeter. The seeds cover the entire surface of the cup at a thickness of about 2 cm. They were placed in direct contact with the measuring head of the spectro-colorimeter, in order to reduce the influence of the parasitic lights on the measured parameters. The measure made was expressed in color stripe as $L^* a^* b^*$, from which the values relating to the color intensity (ΔE), the chroma (ΔC) and the hue angle (H) were extrapolated, according to the formulas:

$\Delta E=((L^*)^2+(a^*)^2+(b^*)^2)^{0.5}$

$\Delta C=((a^*)^2+(b^*)^2)^{0.5}$

$H=\tan^{-1}(a^*/b^*)$

Determination of the centesimal composition of seeds flour and tuber

Determination of the centesimal composition of seeds and tubers of *Sphenostylis stenocarpa* was carried out on samples previously frozen, lyophilized and ground with a laboratory mill IKA M20 (Germany) until obtaining fine and homogeneous flour. Each analysis was carried out in triplicate.

The dry materials of the samples were determined by drying in an oven at 103°C for 24 hours [8].

The ash content of the samples was determined according to the appropriate protocol of standard NF ISO 2171 [9].

The rapid combustion of about 2.3 g of soaked sample of ethanol followed by calcination in a mitten oven (Upsilon Gelman) at 900°C for 150 minutes.

The nitrogen content of the sample (flour) was determined on approximately 200 mg of the sample by the method described by Dumas [10] (1831) using a rapid system of combustion and analyze Rapid N cube® (elementar, Nebraska, USA). The protein content is extrapolated by applying the conversion factor of 6.25.

The fat content was determined by Soxhlet extraction with petroleum ether with the aid of a Soxtherm Gerhardt system according to the Kiger method [11]. Concretely, to 2 g of flour from each sample, are added 50 ml 4N HCl in an Erlenmeyer flask, then mixed. The mixture obtained is placed in a bain-marie at 70°C for 40 minutes. It is then filtered through a double filter paper, pleated of 125 nm moistened. The bottom is washed with distilled water until a neutral pH. The prepared sample is placed in extraction cartridges. Extraction is carried out with 150 ml of petroleum ether, in berlins during 3 h 30 min. The extract obtained is removed from the residual solvent by evaporation, and cooled in the dryer and then weighed.

The starch content was determined using the polarimetric method of Ewers (ISO 10520:1997) [12] after double defecation of the samples with a polarimeter (Bellingham & Stanley Ltd. ADP220, UK).

The amylose content of the starches was evaluated with the method of Morrison et al. [13] as reported by Massaux et al. [14].

Determination of the different fiber families of the samples

The fiber contents were evaluated according to two methods.

The first was consisted sequentially to determine the NDF (Neutral detergent fiber) fractions and ADF (acid detergent fiber) by sequentially washing of the samples. This latter were first subjected to removal of starch by enzymatic hydrolysis in the presence of termamyl alpha-amylase, according to the method of Van Soest et al. [15] as described by Gáspár et al. [16]. The hemicellulose content was calculated by the difference in weight between the ADF and NDF fractions.

The second method consisted in evaluating the total alimentary fiber, including in it, resistant starches and undigested oligosaccharides according to the Prosky method using a Megazyme Kit, such as described by McClaery et al. [17].

Determination of the different families of the proteins of the samples

The protein contents of different families of the samples were evaluated by the Kjeldhal method after sequential extraction of these proteins according to the Osborn et al. [18] as described by Malumba et al. [19].

The salino-soluble proteins (albumins and globulins) were initially extracted with a saline solution (0.5 M NaCl) followed by extraction of the alcohol-soluble proteins (prolamins) thanks to a solution of ethanol containing sodium acetate. The G1 glutins were subsequently extracted with a solution similar to that used for the extraction of prolamines added to beta-mercaptoethanol. The residual glutelins were extracted with the aid of borate buffer pH 10, containing sodium dodecyl sulfate.

The nitrogen contents extracted at the time of the different sequences of extraction were determined by Kjeldhal method and the corresponding proteins deduced with a conversion factor of 6.25. The level of protein extracted in each sequence was calculated by considering the initial quantity of protein in each sample.

A portion of the proteinic extract prepared was purified by dialysis, followed by phase separation for the saline-soluble extracts and a freeze-drying to harvest the isolates of the different proteins.

RESULTS AND DISCUSSION

Morpho-physical characterization of the seeds of the two studied varieties

The **Table 1** below summarizes the results for the morphological and physical characteristics of the seeds of the two main *Sphenostylis stenocarpa* varieties grown in the Democratic Republic of Congo.

From the comparison of the parameters evaluated, it is appear that the produced seeds by the two varieties studied differ in size, specific mass and color. The seeds of the A variety are the less bulky and have however an important specific mass and a weight of 1000 larger grains. This difference indicate a significant difference in the structure of the endosperm of the seeds of the two varieties studied; the seeds of variety A having the thick endosperms. This

characteristic is likely to affect the cooking behavior of these two resources, the softening of seed endosperms during their cooking is dependent on the transfer of water and heat, and on the structure of this one [20].

Table 1: Results of morpho-physical characterization of seeds of *Sphenostylis stenocarpa.*

Parameters	Seeds A	Seeds B
Weight of 1000 seeds (g)	111.91 ± 1.8^a	103.51 ± 3.45^a
Length (mm)	6.94 ± 0.04^a	7.26 ± 0.05^b
Width (mm)	4.80 ± 0.13^a	5.02 ± 0.07^b
Thickness (mm)	4.32 ± 0.12^a	4.52 ± 0.15^a
Voluminal per Seed (mm^3)	75.46 ± 11^a	86.58 ± 14.42^b
Voluminal mass (g/mm^3)	1.48^a	1.2^b
L^*	14.51 ± 0.49^a	14.07 ± 0.80^a
a^*	1.35 ± 0.14^a	3.98 ± 0.20^b
b^*	1.65 ± 0.20^a	3.71 ± 0.53^b
ΔE	14.66 ± 0.2^a	15.09 ± 0.58^a
ΔC	14.82 ± 0.2^a	16.05 ± 0.16^b
H	29.53 ± 0.4^a	31.38 ± 0.36^b

The seeds of the two varieties are also differentiated by the color of their periderm. The seeds of the variety A are slightly more light (L^*), with a less reddish color (a^*), drawing more towards blue (b^*) compared to the seeds of variety B. The intensity of color shows a darker character (ΔC) and a hue angle (H).

Baudoin et al. [21] described seeds of *Sphenostylis stenocarpa* with diameters between 5 and 8 mm. Oshodi et al. [22] and Adewale et al. [23] showed that the varieties of *Sphenostylis stenocarpa* seeds could be distinguished by the color of the pericarp of their seeds. According to this study, the colors of seed pericarp can be white, gray, cream, light brown, deepen brown, violet or black. Such variability may be related to the genotypic characteristics of the varieties studied.

Centesimal composition of the samples

Table 2 shows the centesimal composition of the seed flours of the two varieties studied and those extracted from the tubers of variety B without periderm (FBSP) and with periderm (FBAP).

Table 2: Centesimal composition of samples of seed and tuber of *Sphenostylis stenocarpa.*

Parameters	Seeds A	Seeds B	FBSP	FBAP
MS (%)	92.87	94.39	87.5	87.18
Proteins (%)	22.86 ± 0.10^a	26.72 ± 0.05^b	08.37 ± 0.16^c	10.11 ± 0.08^d
Starch (%)	50.89 ± 0.93^a	46.62 ± 0.29^a	83.93 ± 0.57^b	85.36 ± 0.62^b
Fats (%)	2.08 ± 0.00^a	2.00 ± 0.00^b	0.79 ± 0.01^c	0.50 ± 0.00^d
Ashes (%)	2.25 ± 0.04^a	2.92 ± 0.07^b	1.30 ± 0.02^c	1.04 ± 0.01^d
ADF (%)	8.58 ± 0.05	8.16 ± 0.25	3.05 ± 0.07	2.86 ± 0.03
NDF (%)	16.19 ± 1.31	22.74 ± 1.51	14.28 ± 0.26	10.64 ± 6.57
Hemicellulose (%)	7.61	14.58	11.23	7.78

The centesimal composition of seed flours differs significantly from that of flours extracted from tubers. Seeds are much richer in protein (22.86% and 26.72%), fat (2% and 2.1%) and ash (2.25% to 2.92%) but are poor in starches (46.62% and 50.89%) compared to tuber flours.

The protein content of the seeds of the two varieties differs by about 4%. Evan et al. [24] and Porter [6] showed the protein contents of seed between 21% and 29%. Apata et al. [3] observed a protein content of 20.9%.

The trend obtained by Beckley et al. [25] on several varieties of seeds reveals that the seeds darker are less rich in protein (23.59%) than black seeds (24.06%). This is in contradiction with the results observed in this work.

The variability of the results from one study to another would be fundamentally attributable to the varieties of seeds used, and therefore to genotypes.

Oshodi et al. [22] mention that outside the characteristic genotype of a given variety, environmental conditions would influence the synthesis and accumulation of nutrients within seeds. Since the two varieties are derived from two different ecosystems, some of these differences may depend on the soil and climatic parameters of the growing sites.

It will therefore be important to compare in the future works the characteristics of the seeds of the two varieties grown under similar conditions.

The protein contents of the tubers obtained are lower than those reported by Eromosele et al. [26] (11% to 19%). They are nevertheless close to 10.8% obtained by Ezeuh [27] and reported by Baudoin [21].

Compared with the starch contents, those found in the literature are relative to the total carbohydrate, which doesn't permit to make a reliable comparison. Nevertheless, the starch contents of the seeds are within the range observed by Ekanayake et al. [4]: 24% to 68% carbohydrates and Evans et al. [24]: about 50% carbohydrates. They are also clearly inferior to those of Ezeuh [27] and Klu [28] which obtained respectively the carbohydrate values of 74.1% and 61.6%. The high protein content of variety B appears to be offset by lower starch contents.

Concerning the tubers, the observed contents of starches are in agreement with the value presented by Ezeuh [27]: 86.3% of carbohydrates.

The fat contents of the seeds agree perfectly with that obtained by Oshodi et al. [22]. Indeed, for a study involving six varieties of seeds of *Sphenostylis stenocarpa*, this author obtains an average of 2.10% of fat content. However, these fat contents are higher than those respectively obtained by Duke [29] and Klu [28] (1.2% and 0.5%) and clearly below the values obtained by Beckley et al. [25] (7.89% and 8.13%) for black and brown seeds. The variability of the results would be related to cultural conditions, climatic factors and genetic variability of the varieties investigated.

With regard to tubers, the content obtained is close to that of Ezeuh [27] with a value of 0.6%.

The ash contents of the seeds are of the same order as that (2.4%) obtained by Klu [28]. They are slightly lower than those observed by Beckley et al. [25] on black seeds (3.17%) and brown seeds (2.79%). The flours of the tubers are much less rich in ash; nevertheless they have different values depending on the modalities of preparation of the flours. Flours extracted from tubers without the periderm appear to be slightly richer in ash than whole tubers.

This difference can be due either to a nutritional contribution of the periderm initially less rich in ash, or to a contamination of the sample from the peeled tubers, by minerals during rinsing and grinding.

The NDF content of seeds is significantly higher than 5.7% obtained by Duke [29] for seeds and 1.1% obtained by Ezeuh [27] for tubers such as reported by Baudoin et al. [21]. The samples analyzed are therefore rich in fiber and the variation in the results observed is due to the method of dosing used from one study to another. The methods have evolved significantly over time.

The results of fiber fractionation summarized in the graph (**Figure 1**) show that seeds A are less rich in TDF than seeds B and tubers (FBSP and FBAP). The SDF contents of the seeds are clearly higher than those of the tubers, while the latter are richer in IDF. The high content of insoluble fiber in tubers would be in the majority linked to the presence of resistant starch. A starch digestibility study of tubers of *Sphenostylis stenocarpa* would be desirable for verification of this hypothesis.

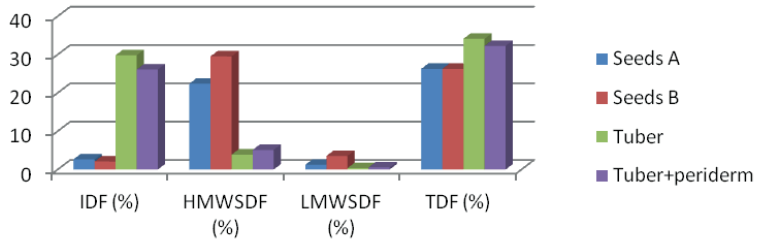

Figure 1: Result of fiber fractionation of samples. F: Insoluble fibers; HMWSDF: High Molecular Weight Soluble Fibers; LMWSDF: Low Molecular Weight Soluble Fibers; TDF: Total Fibers.

According to American Dietetic Association (2008) [30], soluble fibers prolong the sensation of satiety and help to lower the glycemic index of the carbohydrates that it contains, avoid the generation of insulin peaks (particularly harmful in the short and long term). Insoluble fibers increases fecal mass and promote colonic fermentation and then reduce post-prandial blood sugar and pre-prandial cholesterol level [31,32].

One could say that the seeds of *Sphenostylis stenocarpa* would be a good ingredient for the formulation of foodstuffs with anti-bulimic effect while the tubers would be a good ingredient for the foods with laxative effect.

Evaluation of the protein content of seeds and tubers of *Sphenostylis stenocarpa*

Four groups of proteins were extracted from the samples by sequential fractionation according to their solubility and quantified by assay. **Table 3** shows the protein content of these different groups in the *Sphenostylis stenocarpa* samples analyzed.

According to **Table 3**, in the seeds as in the tubers of *Sphenostylis stenocarpa*, the saline-soluble proteins constitute the group of the most representative proteins.

Table 3: Protein content of the samples..

	Average content (%)			
Proteins	**Seed A**	**Seed B**	**FBSP**	**FBAP**
Albumins	8,05	1,16	43,61	55,98
Globulins	40,77	37,99	3,11	3,66
Alcohol-solubles	1,66	1,65	0,96	0,40
Glutelins G1	0,39	0,33	1,31	0,49
Glutelins G2+G3	30,10	36,94	12,90	11,67
Residual base	3,09	2,85	2,53	2,7
Total extracted	80,97	78,07	61,89	72,20

This group of proteins is consisted mostly of globulins in the seeds, whereas the albumins dominate in the tubers.

Ajibola et al. [33] after extracting of the saline-soluble proteins from the seeds and purification by dialysis, and then protein assay by the method of Lowry found values of 10.23% and 39.15% respectively for albumins and globulins. These values are close to those observed in the present work.

Seeds and tubers contain very few prolamins (alcohol-soluble). The prolamin content of the seeds is relatively higher than in the tubers.

The glutelins, particularly G2 and G3, constitute the second most representative group of proteins in the seeds and tubers of *Sphenostylis stenocarpa*. Seeds, however, appear to be much more rich in glutinins than tubers. By comparing the seeds of the two varieties, there is a significant difference between the levels of globulins, with the seeds of variety A which is richer in this component. On the other hand, the seeds of variety B appear to be richer in glutenins (G2+G3) than those of variety A.

The predominant content of globulin in seeds of *Sphenostylis stenocarpa* is consistent with earlier observations according to which the seeds of most legumes are constituted of globulin (45%-70%) followed by gluthelins [34]. According to Sathe [34], the protomer of globulin 7S would be the major component of legume seed proteins. Unfortunately, there is no work today which has carried out a complete sequential extraction of the different families of *Sphenostylis stenocarpa* proteins. Only Ajibola et al. [33] extracted the saline-soluble proteins which they subsequently fractionated into albumin and globulin by dialysis. It would be useful for future work to validate the observations presented in this work, while attempting to improve the recovery efficiency of proteins by sequential solubilisation.

CONCLUSION

The varieties of *Sphenostylis stenocarpa*, although being the same species, are characterized by considerable differences, sometimes of nature very controversial. In the seeds as in the tubers, the nutritional profiles appear very interesting in term of human food or livestock. The *Sphenostylis stenocarpa* come out of the circle of the plant species neglected and under-exploited can contribute to reduce the prevalence of the famine and to reinforce the food security in Africa. With a view to take advantage fully of the nutritional potential of this resource, it is necessary to survey in toxicology, the likely anti-nutritional factors and to arrange their elimination.

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