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Effect of Germination on Proximate, Available Phenol and Flavonoid Content, and Antioxidant Activities of African Yam Bean (*Sphenostylis stenocarpa*)

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***Abstract*—**The work studied the effect of germination on proximate, phenol and flavonoid content and antioxidant activities (AOA) of African Yam been (AYB). Germination was done in controlled dark chamber (100% RH, 28^{o}C). The proximate, phenol and flavonoid content and antioxidant activities before and after germination were investigated. The crude protein, moisture, and crude fiber content of germinated AYB were significantly higher ($P<0.05$) than that of ungermianated seed, while the fat, Ash and carbohydrate content of ungerminated were higher than the germinated seed. Germination increased the phenol and flavoniod content by 19.14% and 14.53% respectively. The results of AOA assay showed that the DPPH, reducing power and FRAP of germinated AYB seed gave high values: 48.92 ±1.22 µg/ml, 0.75± 0.15µg/ml and 98.60±0.04 µmol/g while that of ungerminated seed were: 31.33µ/ml, 0.56±1.52µg/ml and 96.11±1.13µmol/g respectively. Germinated AYB has phytochemicals with potential AOA for disease prevention.

***Keywords*—**Antioxidant, flovonoid, germination, phenol.

I. Introduction

ANTIOXIDANTS are radical scavengers that inhibit or slow down the oxidation of other molecules by blocking the propagation of oxidizing chain reactions that lead to degenerative diseases such as cancer, inflammation, anaemia, diabetes, neuro-degeneration, cardiovascular and ageing [1], [2]. Phenols and flavonoids, which are excellent antioxidants, can scavenge reactive oxygen and nitrogen species thereby preventing the onset of oxidative diseases in the body.

The use of natural antioxidants is on the increase due to the carcinogenic effect of synthetic antioxidants, such as butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA), have on humans [3]. Several researchers have reported that antioxidants of plant origin can protect human body against oxidative stress [4].

With the aim of improving the bioactive compounds in legumes, preparation techniques have been developed to significantly raise the bioavailability of their antioxidants. Such techniques include germination, during which some seed reserve materials are degraded and used for synthesis of new cell constituents, causing significant changes in the biochemical, nutritional and sensory characteristics of the modified legumes [5].

Sprouting modifies the phytochemical content into antioxidants that act as protective factors against oxidative damages in the human body [1]. African yam bean is one of the underutilized legume in Africa particularly in Nigeria, Togo and Cameroun. This herbaceous climbing vine produces ellipsoid, rounded or truncated seeds, which show considerable variation in size and colour, varying from creamy – white or brownish – yellow to dark brown. Both the seeds and leaves of the plant are edible. The plant also produces tubers, which can be cooked and eaten. They are important sources of starch and protein [6]. There is scarcity of studies on the effect of germination on the antioxidant activity of African yam bean extract. Therefore, the aim of this work was to evaluate the effect of germination on proximate composition total phenols and flavonoid compounds and antioxidant activity of African yam bean.

II. Materials and Methods

Dried African yam bean seed (*Sphenostylis stenocarpa*) were purchased from Ogbete Main Market in Enugu State – Nigeria. The samples were contained in plastic sealed and stored before germination.

A. Germination Process

A 300 g of African yam bean was soaked in 1 litre of water containing 0.7% sodium hypochlorite solution for 30 minutes at room temperature (28^{o}C). The water was drained off, and re-soaked in distilled water for 5 hours and the water also drained. The hydrated seeds were placed under wet muslin cloth and left to germinate for 3 days at room temperature (28^{o}C) without direct contact with sunlight [7]. The sprouted seeds were oven dried (Gallenkemp 1H – 100 model, UK) at 60 ^{o}C for 4 hours and milled to pass through 0.18 mm sieve to obtain the flour which was packaged. The non sprouted seeds were ground, sieved and packaged. This served as control.

B. Extraction of the Seed

A 200 g of both the sprouted and non sprouted flour samples were defatted separately by stirring with 100 ml of 70% acetone at 25^{o}C for 24 hours and filtering through Whatman No. 4 filter paper, following the method described previously [8]. The residues were further defatted with an additional 50 ml of 70% acetone, as described above for 3 hr. The solvent of the combined extract was evaporated under

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reduced pressure, using a rotary vacuum evaporator (RE 300, Yamato, Tokyo, Japan) at 40°C and the remaining water was removed by Lyophilization (4KBTxL-75; Virtis Benchtop K, New York, USA). The obtained dry powder was stored in an air tight polythene bag at 0°C until it was used.

C. Determination of Proximate Composition

The moisture, crude protein, fat, fibre, and ash content of the samples were determined in triplicates according to standard method described elsewhere [9]. The carbohydrate content was determined by difference.

D. Determination of Total Phenol

Total phenol content of the sample was determined using the method described previously [10]. A 50 µL of the sample extract was put in test tubes and the volume was made up to 500 µL using distilled water. Then 250 µL of folin-ciocalteu reagent was added into test tube followed by 1.25 ml of 20% sodium carbonate solution. The tube was vortexed before being incubated in the dark for 40 minutes. Absorbance was read at 725 nm using spectrophotometer.

E. Determination of Total Flavonoid

Aluminium chloride colorimetric method was used for flavonoids determination [11]. The Sample extract (0.5 ml of 1:10 g/ml) in methanol were mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The mixture remained at room temperature (28°C) for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA). The calibration curve was prepared with quercetin solutions at concentrations 12.5 to 100 g ml^{-1} in methanol. The concentration of the sample extract was then extrapolated from the standard curve drawn (Absorbance against concentration).

F. Determination of DPPH free Radical Scavenging Activity

DPPH scavenging activity was carried out by the method described elsewhere [12]. A 250 µg/ml of African Yam bean seed extract with methanol was dissolved in DMSO (dimethyl sulfoxide) and pipette into test tubes in triplicates. Then 5 ml of 0.1 M ethanol solution of DPPH (1,1 Diphenyl-2-picrylhydrazyl) was added to each of the test tubes and were shaken vigorously. It was allowed to stand at 35°C for 20 minutes. The control was prepared without any extracts. Methanol was used for base line corrections in absorbance (OD) of sample and measured at 517 nm. A radical scavenging activity was expressed as 1 % scavenging activity and was calculated by the following formula.

$$\text{Radical scavenging activity \%} = \frac{\text{OD control} - \text{OD sample}}{\text{OD Control}} \times 100$$

G. Reducing Power Assay

Reducing power of the sample extract was determined according to the procedure described previously [13]. Aliquots (2.5 ml) of sample extract in phosphate buffer (0.2 M phosphate buffer, pH 6.6) was added to 2.5 ml of potassium ferricyanide (10 mg/ml) and the reaction mixture incubated at 50°C for 20 min. Trichloroacetic Acid (TCA) (2.5 ml of 100 mg/ml solution) was then added to the reaction mixture, vortexed and centrifuged at 1000 rpm for 10 min. The resultant supernatant (2.5 ml) was mixed with an equal volume of distilled water and 0.5 ml of ferric chloride was added (1 mg/ml solution). Absorbance was measured spectrophotometrically at 700 nm against ascorbic acid as standard and higher absorbance of sample indicates greater reducing power.

H. Ferric Reducing/Antioxidant Power (FRAP) Assay

Ferric reducing antioxidant power of the sample extract was determined as described elsewhere [14]. This method is based on the ability of the sample to reduce Fe^{+3} to Fe^{+2}. FRAP reagent (900 µl), prepared freshly and incubated at 37°C, was mixed with 90 µl of distilled water and 30 µl of seed extract of methanol (for the reagent blank). The seed extract and reagent blank were incubated at 37°C for 30 min in a water bath. The final dilution of the test sample in the reaction mixture was 1/34. The FRAP reagent contained 2.5 ml at 20 mmol/l 2,4,6-tripyridyl-triazine (TPTZ) solution in 40 mmol/l HCl plus 2.5 ml of 0.3 mol/l acetate buffer (pH 3.6). After incubation for 6 min at room temperature, reduction of TPTZ to the ferrous complex formed a blue colour was measured at a wavelength of 593 nm. $FeSO_4$ was used as standard.

III. Statistical Analysis

Data was subjected to analysis of variance using the statistical package for social science (SPSS), version 15.0. Results were presented as mean ± standard deviations. One way analysis of variance (ANOVA) was used for comparison of the means. Differences between means were considered to be significant at $p<0.05$ using the Duncan Multiple Range Test. Values are average of triplicate experiments ± standard deviation.

IV. Results and Discussion

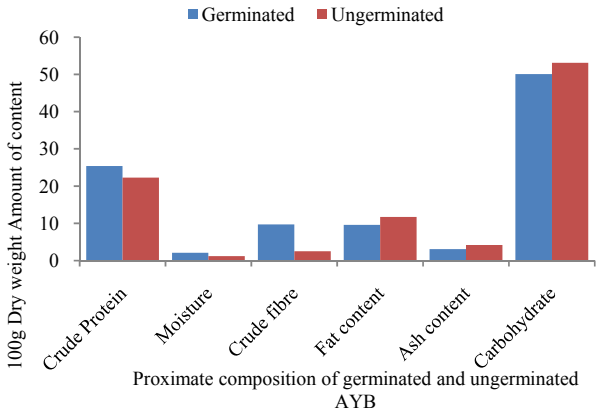

Fig. 1 The effect of germination on the proximate composition of African Yam Bean

The results of the proximate composition of both the germinated and ungerminated AYB are shown in Fig. 1. The crude protein, moisture and crude fibre contents of the germinated seed were significantly higher ($p<0.05$) than that of the ungerminated seeds while their fat content, Ash content and carbohydrate were lower. The observed increase in the crude protein content of germinated seed might be attributed to a net synthesis of enzymes (e.g. protease) by germinating the seed [15]. The observed decrease in the total carbohydrate after germination might be due to increase in α-amylase activity. The α-amylase breaks down complex carbohydrate to simpler and more absorbable sugars which are utilized by the growing seedlings during the early stages of germination. This is in agreement with previous report [16] which observed a decrease in carbohydrate content after germination. Also the observed decrease in fat contents of the germinated seeds corroborated with former observation made elsewhere [17] who observed a decrease in fat after germinating bambara groundnuts. The decrease in fat content of germinated seeds might be due to the increased activities of the lipolytic enzymes during germination. They hydrolyzed fats to simpler products which can be used as source of energy for the developing embryo. Thus decreased fat content implies an increased shelf-life for the germinated seeds compared to the ungerminated ones.

TABLE I
TOTAL PHENOL AND TOTAL FLAVONOID CONTENT OF AFRICAN YAM BEAN

Treatment	Total phenol (mg/100g dry weight)	Total flavonoid (mg/100g dry weight)
Germinated African yam bean	117.08±0.03[a]	68.31±1.12[a]
Non Germinated African yam bean	98.27±0.11[b]	59.64±0.01[b]

Value are means ± SD; n = 3. Mean values followed by different letters in a column are significantly different ($p<0.05$).

A. Total Phenol Content

Phenol and other phytochemical found in fruits, vegetables and legumes are bioactive compounds capable of neutralizing free radicals and many play a role in the prevention of certain diseases [18].

Functional food and nutritional supplements eliminates certain risks and have a preventive effect that is based on the therapeutically and regulatory effect of nutrients [19]. Phenolic compounds contribute to the overall antioxidant activities of the plant foods by acting as free radical terminators. Total phenolic content (TPC) of the extracts of germinated and non germinated African yam bean (AYB) are shown in Table I. The TPC in germinated AYB is higher ($p<0.05$) than that of non-germinated AYB. This increase in the amount of phenolic compound after germination is in accordance with former observation [5] which indicates that germination modifies the quantity and quality of phenolic compounds in legumes. Also, the work done previously [20] is in concordance with this present work, they germinated lupin seeds (*Lupinus angustifolius L.*) and observe 46% increase in total phenols.

B. Total Flavonoid Content

Researches have shown that intake of foods rich in flavonoid protects human against diseases associated with oxidative stress. The mechanisms of action of flavonoids are through free-radical scavenging or chelating process and protection against oxidative stress [21]. The flavonoid contents of the extracts of germinated AYB (68.31 mg/100 g of dry weight) were higher ($P<0.05$) than that of non germinated AYB (59.64 mg/100 g). Their findings were attributed to the biochemical metabolism of seeds during germination, which might produce some secondary plant metabolites such as anthocyanins and flavonoids [22].

TABLE II
ANTIOXIDANT ACTIVITY OF AFRICAN YAM BEAN

Treatment	DPPH (µg/ml)	Reducing power (µg/ml)	FRAP (µmol/g)
Germinated African yam bean	48.92±1.22[a]	0.75±0.15[a]	98.60±0.04[a]
Non Germinated African yam bean	31.33±1.14[b]	0.56±1.52[b]	96.11±1.13[b]

Value are means ± SD; n = 3. Mean values followed by different letters in a column are significantly different ($p<0.05$).

C. DPPH Assay

Free radicals are involved in many disorders like neurodegenerative diseases, cancer and diabetes [18]. Antioxidants through their scavenging power are useful for the management of those diseases. Radical scavenging activity using DPPH has been use to survey the antioxidant activity of agricultural produce [23]. The free radical scavenging activity of germinated and non germinated AYB was tested by measuring their ability to quench the DPPH radical (Table II). The results showed that germinated AYB had the higher DPPH free radical scavenging ability (48.92 µg/ml) than non germinated AYB (31.33 µg/ml).

The high scavenging property of germinated AYB may be due to hydroxyl groups existing in the phenolic compounds' chemical structure that can provide the necessary component as a radical scavenger. DPPH is a stable free radical with adsorption maximum at 517 nm. It does this adsorption when accepting an electron [24]. Therefore, germinated AYB could contain some substances which are electron donors that reacted with free radicals to convert them to more stable products and block the radical chain reaction.

D. Reducing Power Assay

Table II presents the reducing power of germinated and non germinated AYB. The results showed that the reducing power of germinated AYB (0.75±0.15 µg/ml) was greater than that of none germinated (0.56±1.52 µg/ml). Reducing power assay is used to evaluate the ability of natural antioxidants to donate electrons. It has been accepted that the higher the absorbance at 700 nm, the greater the reducing power [25]. Samples with higher reducing power have better abilities to donate electrons and free radicals to form stable substances, thereby interrupting the free radical chain reactions [26]. The result from this reducing power assay indicated that germinated AYB which has a higher reducing power than non germinated AYB will have a better ability to donate electron which is

related to the antioxidant activity. This finding is in agreement with the work done previously [27] which reveals that germination enhances the antioxidant capacity of chick pea. It also agrees with previous work [5]. In their work, they studied the effects of varying germination conditions for bean, lentils and pea, at semi-pilot scale, on bioactive compounds and indicated that peas and beans undergo significant increases in antioxidant activities after germination, whereas lentils show a decrease.

E. Ferric Reducing/Antioxidant Power (FRAP) Assay

The FRAP assay measures the antioxidant effect of any substance in the reaction medium. The method is based on the ability of the sample to reduce Fe^{+3} to Fe^{+2} ions. The reducing power of germinated AYB (98.60±0.04µmol/g) was found to be higher than that of non germinated AYB (96.11±1.13µmol/g). The presence of higher antioxidant in the germinated sample caused a higher reducing power compared to the non-germinated.

V. Conclusion

Based on the findings obtained in this study, germination process caused increase in proximate, total phenol and flavonoid content as well as increased the anti oxidant properties of African yam bean .

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