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Full Length Research Paper

Evaluation of the Proximate Composition and Antinutritional Properties of African Yam Bean *(Sphenostylis sternocarpa)* Using Malting Treatment

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Abstract

The effect of malting on the proximate composition and anti-nutritional properties of African yam bean (AYB) (Sphenostylis sternocarpa) was investigated. Proximate and anti-nutritional analyses were carried out on three samples of AYB namely: raw dehulled bean flour (R.S), soaked (24h) and malted (24, 48, 72, 96)h dehulled bean flour and soaked (48h) and malted (24, 48, 72, 96)h dehulled bean flour. From the results of the proximate composition, it showed that protein had the highest increase in value for sample soaked for 48 hours and malted for 96h (24.38%) while ash, fat, moisture content, crude fibre, for samples soaked for 24h and malted for 24h-96h reduced from 3.23-3.19%, 1.64-1.59%, 9.43-8.64%, 5.31-5.12% respectively, while samples soaked for 48h and malted 24h-96h reduced also from 2.93-2.91%, 1.61-1.57%, 9.41-8.85%,5.17-4.93% respectively. Also, from the results obtained, the anti-nutritional properties, phytate, alkaloid, saponin, stachyose, raffinose, haemagglutinin, oxalate, tannin, phenol, hydrogen cyanide, trypsin inhibitor for samples soaked for 24h and malted for 24-96h reduced from 0.363-0.346%, 0.43-0.37%, 0.61-0.49%, 0.309-0.267%, 0.077-0.067%, 138.73-58.70HUI/g, 0.299-0.207%, 0.230-0.121%, 0.113-0.111%, 22.66-10.31mg/kg, and 22.09-11.64TUI/g respectively and for samples soaked for 48h and malted for 24h-96h reduced from 0.350-0.339%, 0.41-0.35%, 0.49-0.39%, 0.275-0.235%, 0.069-0.059%, 93.83-33.53HUI/g, 0.229-0.199%, 0.208-0.098%, 0.112-0.080%, 19.88-9.09mg/kg and 18.27-8.70TUI/g respectively. The nutritional quality was improved for both the proximate and anti-nutritional composition as a result of these treatments. Thus, it could be used in food formulations.

Keywords: *African yam bean, Anti-nutritional properties, Malting, Soaking, Proximate properties*

Introduction

African yam bean (*Sphenostylis stenocarpa* (Hochst. ex A. Rich.) Harms) is a perennial climbing bush generally grown as an annual crop. It belongs to the family *fabaceae* and genus *sphenostylis* (Potter and Doyle, 1994). It is one to three meters high. The plant flowers after 90 days. Pods mature from 140 to 210 days and the edible tubers, which look like elongated sweet potatoes, are ready to harvest from 150 to 240 days after sowing (Ecocrop, 2009).

African yam bean (*Sphenostylis stenocarpa*) is an underutilized food legume crop in the tropics; not as popular as other major food legumes (Moyib *et al*., 2008). It is a typical African plant grown in most parts of the hot and humid tropical regions at middle and low altitudes and more specifically in southern Nigeria (Raemakers, 2001). Nigeria is very significant for African yam bean production (Potter, 1992) where extensive cultivation has been reported in the Eastern (Abbey and Berezi, 1998) Western, and Southern (Saka and Ajibade, 2004) parts of Nigeria. It is called different names in different countries; the local names in Nigeria include "Girigiri" (Hausa), "sese" (Yoruba), "Ijinji", "Odudu", "Azuma" (Igbo), "Nsuma" (Ibibio) (Amihud, 1992).

The protein content in AYB seeds ranges between 21 and 29% and in the tubers of AYB the protein is about 2 to 3 times the amount in potatoes (Uguru and Madukaife 2001; Okigbo 1973) and higher than those in yam and cassava (Amoatey *et al*., 2000). Moreover, the amino acid values in AYB seeds are higher than those in pigeon pea, cowpea, and bambara groundnut (Uguru and Madukaife, 2001). African yam bean is rich in minerals such as potassium, phosphorous, magnesium, calcium, iron and zinc but low in sodium and copper (Edem *et al.*, 1990). The seeds contain tannins, trypsin inhibitors, hydrogen cyanide, saponins and phytic acid (Akinmutimi *et al*., 2006). Processing such as heating, soaking or fermenting has been used to lower anti-nutritional factors and improve their nutritional value (Onyeike *et al*., 1995).

Sphenostylis stenocarpa is cultivated for its tubers and for its seeds. It is mainly used as food but can be used to feed animals. It has been suggested that the legume may be prepared into "moi-moi" and can also be toasted in a hot frying pan, and testa removed to produce a toasted cotyledon which may be eaten as a snack alone or with fresh coconut or palm kernels (Ishiwu and Onyeji, 2004). In Nkwanta district, the Konkombas mill the dry seeds into flour, process into paste, then wrap in plantain leaves, boil and eat as

"turbani" (Okigbo, 1973). The flour may also be mixed with cassava flour, cooked into a paste and eaten with soups. Cooked beans are made into a sauce and eaten with ''gari'' - a cassava product. Some of the farmers reported that the water drained after boiling the beans may be drunk by lactating mothers to increase their milk production. AYB is used to replace cowpea in most food preparations, especially during the lean period when food is scarce among the rural farmers. It can also be used as a protein supplement.

Some of the problems associated with African yam bean is the characteristic hardness of the seed coat (Oshodi *et al.*, 1995) which makes a high demand on the energy cost as a result of the long cooking time, the agronomic demand for stakes and the long maturation period (Okpara and Omaliko, 1997). The seeds also contain anti-nutrients like tannins, trypsin inhibitors, hydrogen cyanide, saponins and phytic acid which could bind with metals and hinder the absorption of nutrients into the body and secondary metabolites (NRC, 1979). The photoperiodic sensitivity of African yam bean seems to compound the above disadvantages as it confines the cultivation and production of the crop to one season in the year. Another difficulty is in the process of dehulling (Eke and Akobundu, 1993).

The objective of this study therefore, is to determine the effect of malting on the proximate and anti-nutritional properties of African yam bean and also its effect on the dehulling of the seed coats.

Materials and Methods

African yam bean seeds (*Sphenostylis stenocarpa*) used for this research work were purchased from the local traders at *Ekeonunwa* market in Owerri, Imo State, Nigeria. The chemicals and equipment used were of analytical grade and were obtained from the Department of Food Science and Technology, Federal University of Technology, Owerri and the central laboratory of National Roots Crops Research Institute, Umudike, Umuahia in Abia State, Nigeria.

Sample Preparation

The purchased dry seeds (African Yam bean) were cleaned and sorted to remove extraneous materials and damaged seeds. About 2kg of the seeds were divided into three different portions. Two portions were soaked in 2 litres of water each (in different containers, to give a bean: water ratio of 1:2) for 24 hours and 48 hours, and were malted for 24 hours, 48 hours, 72 hours and 96 hours. They were then dried at 60ºC for 8 hours in a carbolite electric oven.
The third portion (raw sample) was soaked for a few minutes to release the hulls from the cotyledon and sundried. The dried dehulled seeds were ground into fine flour using attrition mill, it was labeled and stored in an air tight container to be further analysed.

Proximate composition analysis

The proximate analysis of the samples were carried out using the methods as follows:

Moisture Content Determination

This was carried out by the gravimetric method (A.O.A.C, 1990). Five grams (5g) of the sample was weighed into a previously weighed moisture can. The sample in the can was dried in the moisture extractor at 105^{O}C for 3 hours. It was cooled in a dessicator and weighed. It was then returned to the oven for further drying. Drying, cooling and weighing were done repeatedly at an hour interval until there were no further diminutions in the weight (i.e. a constant weight was obtained). The weight of moisture loss was calculated and expressed as a percentage of the weight of sample analysed.

$$\% \text{ moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

where, W_1 = Weight of empty can
W_2 = Weight of empty can + sample before drying
W_3 = Weight of can + sample dried to a constant weight
% total solid (Dry matter) = 100 - % moisture content

Ash Content Determination

This was done by furnace incineration gravimetric method (James, 1995). Five grams (5g) of the processed sample was measured into a previously weighed porcelain crucible. The sample was burnt to ashes in a muffle furnace at 550^{O}C. When completely ashed, it was cooled in a desiccator and weighed. The weight of ash obtained was calculated by difference and expressed as a percentage of the weight of sample analysed.

$$\% \text{ Ash content} = \frac{W_2 - W_1}{\text{Weight of sample}} \times \frac{100}{1}$$

where, W_1 = weight of empty crucible
W_2 = weight of crucible + Ash

Crude Fibre Determination
The Weende method (James, 1995) was employed. Five grams (5g) of the processed sample was boiled in 150ml of 1.25% H_2SO_4 solution for 30min under reflux. The boiled sample was washed in several portions of hot water using a two-fold muslin cloth to trap the particles. The residue was returned to a flask and boiled again in 150ml of 1.25% NaOH for another 30min under the same condition. After washing in several portion of hot water, the sample was allowed to drain before it is transferred to a weighed crucible where it was dried in the oven at 105^{O}C to a constant weight. It was thereafter taken to a muffle furnace in which it was burnt until only ash was left of it. By difference, the weight of fibre was obtained and expressed as a percentage of the weight of sample analysed.

$$\% \text{ crude fibre} = \frac{W_2 - W_3}{\text{Weight of sample}} \times \frac{100}{1}$$

where, W_2 = weight of crucible + sample after boiling, washing and drying.
W_3 = weight of crucible + sample ashing.

Fat Determination
This was done using the continuous solvent extraction gravimetric method using a soxhlet apparatus, as described by Pike (2003). One gram (1g) of sample was wrapped in a previously weighed porous paper (Whatman No 1 filter paper) and placed in a clean dry soxhlet reflux flask. The flask was mounted unto an extraction flask containing 300ml of normal hexane. The upper end of the reflux flask was connected to a water condenser.On heating the extraction flask with a non-luminous heat source (hot plate), the solvent boiled, vaporized and condensed into the reflux flask and covered the wrapped samples. The sample remained in contact with the solvent until the reflux flask filled up and siphoned over thereby carrying extracted oil (fat) down to the boiling flask. The cycle of vaporization, condensation, extraction and reflux siphon was allowed to go on repeatedly for about fourteen times (4h). The defatted wrapped samples were carefully removed (with the aid of pair of forceps) and dried in the oven at 100^{O}C for 30min after which they were cooled in a desiccator and weighed. By difference, the weight of oil (fat) lost was calculated and expressed as a percentage of the sample weight.

$$\% \text{ fat} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

where, W_1 = weight of empty filter paper
W_2 = Weight of paper + sample before defatting
W_3 = weight of paper + sample after defatting

Crude protein determination
This was done by the Kjeldahl method described by Chang (2003). The total nitrogen was determined and multiplied with factor 6.25 to obtain the protein content. Half gram (0.5g) of the sample was mixed with 10ml of concentrated H_2SO_4 in a digestion flask. A tablet of selenium catalyst was added to it before it was heated under a fume cupboard until a clear solution was obtained (i.e. the digest). The digest was diluted to 100ml in a volumetric flask and used for the analysis. 10ml of the digest was mixed with equal volume of 45% NaOH solution in a Kjeldahl distillation into 10ml of 4% buric acid containing three drops of mixed indicator (bromocressol green/methyl red). A total of 50ml of distillates was collected and titrated against 0.02N EDTA from green to a deep red end point. A reagent blank was also digested, distilled and titrated. The nitrogen and protein contents were calculated using the formula below:

$$\% \text{ protein} = \% N2 \times 6.25$$

$$\% N_2 = \frac{100}{10} \times \frac{N \times 14}{1000} \times \frac{Vt}{Va} \text{ T-B}$$

where, W = Weight of sample (0.5g)
Vt = Total digest volume (100ml)
Va = Volume of digest analysed (10ml)
T = Sample titre value
B = Blank titre value

Carbohydrate Determination
This was determined by the difference method (James, 1995). The calculation is given by the equation:

% Carbohydrate = $100 – (M+P+F_1+A+F_2)$
P = Protein
F_1 = Fat
A = Ash
F_2 = Fibre

Determination of anti-nutritional factors
Determination of Tannins
This was determined by Folin Denis colometric method. Five grams (5g) of the flour sample was put inside a volumetric flask and 50ml of distilled water was dispensed inside the volumetric flask. The mixture was shaken for 30 minutes at room temperature and filtered to obtain the extract. A standard tannic acid solution was prepared, 2ml of the standard solution and equal volume of distilled water were dispersed into a separate 50ml volumetric flasks to serve as a standard and reagent blank respectively. Then 2ml of each of the sample extracts was put in their respective labeled flask.

The content of each flask was mixed with 35ml distilled water and 1ml of the Folin Denis reagent was added to each. This was followed by 2.5ml of saturated Na_2CO_3 solution. Therefore, each flask was diluted to the 50ml mark with distilled water and incubated for 90minutes at room temperature. Their absorbance was measured at 760nm in a spectrophotometer with the reagent blank at zero.

The tannin content was calculated as shown below:

$$\% \text{ Tannin} = \frac{100}{W} \times \frac{au}{as} \times C \times \frac{Vt}{Va}$$

Where, W = weight of sample
au = absorbance of test sample
as = absorbance of standard tanning solution
C = Concentration of standard tannin Solution
Vt = Total volume of extract
Va = Volume of extract analysed

Determination of saponins
This was done by the double solvent extraction gravimetric method (Harborne, 1973). Five grams (5g) of the sample was mixed with 50ml of 20% aqueous ethanol solution and incubated for 12 hours at a temperature of $55^{O}C$ with constant agitation. After that, the mixture was filtered through Whatman No 42 grades of filter paper. The residue was re-extracted with 50ml of the ethanol solution for 30 minutes and the extracts weighed together.

The combined extract was reduced to about 40ml by evaporation and then transferred to a separating funnel and equal volume (40ml|) of diethyl ether was added to it. After mixing well, there was a partition and the other layer was discarded while the aqueous layer was reserved. This aqueous layer was re-extracted with the ether after which its pH was reduced to 45 with dropwise addition of dilute NaOH solution.

Saponin in the extract was taken up in successive extraction with 60ml and 30ml portion of normal butanol. The combine extract (ppt) was washed with 5% NaCl solution and evaporated to dryness in a previously weighted evaporating dish. The saponin was then dried in the oven at $60^{o}C$ (to remove any residual solvent) cooled in a desiccator and re-weighed. The saponin was determined and calculated as a percentage of the original samples.

$$\% \text{ saponin} = \frac{W2 - W1}{W} \times \frac{100}{1}$$

where, W = weight of sample used
W1 = weight of empty evaporation dish
W2 = weight of dish + saponin extract

Determination of alkaloids

The alkaline precipitation gravimetric method (Harbone, 1973) was used. Five grams (5g) of the sample was dispersed in 100ml of 10% acetic acid in ethanol solution. The mixture was shaken well and allowed to stand for 4 hours at room temperature and shaken every 30minutes. At the end of this period, the mixture was filtered through Whatman No 42 grade of filter paper.

The filtrate extract was concentrated by evaporation, to a quarter of its original volume; the extract was treated with drop wise addition of concentrated NH_3 solution to precipitate the alkaloid. The dilution was done until the NH_3 was in excess. The alkaloid precipitate was removed by filteration using weighed Whatman No 42 filter paper. The paper was dried at 60^oC and re-weighed after cooling in a desiccator. The weight of alkaloid was determined and expressed as a percentage of the sample.

$$\% \text{ Alkaloid} = \frac{W_2 - W_1}{\text{Weight of sample}} \times \frac{100}{1}$$

where, W_1 = weight of empty filter paper
W_2 = weight of filter paper + alkaloid precipitate

Determination of Hydrogen Cyanide (HCN)

This was determined by alkaline pikrate colourimeter method by Balagopalan *et al.* (1988).
One gram (1g) of the sample was dispersed in 50ml of distilled water in a 25ml conical flask. An alkaline pikrate paper was hung over the sample mixture and the blank in their respective flasks.

The set up was incubated overnight and each pikrate paper was eluted into a 60ml of distilled water. A standard cyanide solution was prepared and diluted to a required concentrate. The absorbance of the eluted sample solution and that of the standard were measured spectrophotometrically at 540nm wavelength with the reagent blank at zero.

The cyanide content was determined by the formula shown below:

$$\text{HCN mg/kg} = \frac{1000}{W} \times \frac{au}{as} \times C \times D$$

where, W = Weight of sample analyzed
au = absorbance of test sample
as = absorbance of standard HCN solution
D = Dilution factor where applicable
C = concentration of the standard in mg/dl

Determination of Trypsin Inhibitor

This was done using the spectrophotometric method, described by Armtfield *et al*., (1985).
Five grams (5g) of the test sample was dispersed in 50ml of 0.5m NaCl solution and stirred for 30 minutes at room temperature. It was centrifuged and the supernatant filtered through Whatman No 42 filter paper. The filtrate was used for the assay. Standard trypsin was prepared and used to treat the substrate solution (N-benzol-D1-arginine-p-anilide; BAPA). The extent of inhibition was used as a standard for measuring the trypsin. In the tube containing 2ml of extract, 10ml of the substrate (BAPA) was added. Also the 2nd part of the standard trypsin solution was added in another test tube containing only 10ml of the substrate. The latter served as the blank.
The content of the tubes were allowed to stand for 30 minutes and then the absorbances of the solution were measured spectrophotometrically at 410nm wavelength. One trypsin activity unit inhibited is given by an increase on 0.01 absorbance unit at 410nm.

$$\text{Trypsin unit inhibited/100g} = \frac{Au}{As} \times 0.01 \times F$$

where, Au = Absorbance of standard (uninhibited sample)

$$F = \text{Experimental factor given as } \frac{Vf}{Va} \times \frac{1}{W}$$

where, Vf = Total volume of extract
Va = Volume of extract analysed

W = Weight of sample analysed

Determination of Haemaglutinin

This was determined using the spectrophotometric method of Armtfield *et al.* (1985).
Half of a gram of the sample was mixed with phosphate buffer solution (50ml) and filtered to obtain the extract used in the analysis. An aliquot (1ml) of the extract from each sample was added to a test tube containing 9ml of suspended trypsinated rabbit red blood cells. A control test tube contained the suspended cells but without extract. The tubes were allowed to stand at room temperature for 10 minutes before their respective absorbances were measured in a spectrophotometer of 510nm wavelength. The amount of agglutinin in the test sample was expressed as the number of units of agglutinin observed per gram of the test sample.

$$HUI/g = \frac{1}{W} \times au - as \times \frac{Vf}{Va}$$

HUI/g = Haemagglutinin units per gram
W = weight of sample used
Vf = Total extract volume
Va = Volume of extract

Determination of Oligosaccharides

The authrone method for sugar analysis as desribed variously was used (Pearson, 1976; James, 1995). Five grams (5g) of the sample was boiled in 100ml of bench hydrochloric acid (2M HCl) solution until it tested negative to starch iodine test. The hydrolysed sample was filtered through Whatman No 42 filter paper using more acid solution to wash the hydrolysate until 100ml filtrate was obtained. An aliquot (1ml) of the filtrate was mixed with 6ml of authrone reagent in a test tube. Both the samples and standard were corked and boiled in a water bath for 10 minutes. After cooling to room temperature, they were filtered and the respective absorbances were read in a spectrophotometer at a wavelength of 620nm. A reagent blank was used to set the instrument to zero. The total sugar was calculated and the factors 0.04 and 0.01 were used to multiply the total sugar to obtain the stachyose and raffinose contents respectively.

$$\text{Thus, \% Total sugar} = \frac{100}{W} \times \frac{au}{as} \times \frac{C}{100} \times \frac{Vf}{Va}$$

Where, W = Weight of sample analysed
au = Absorbance of test sample
as = Absorbance of standard sample
Vf = Total volume of extract
Va = Volume of extract analysed
C = Concentration of standard sample
% Stachylose = % Total sugar x 0.04
% Raffinose = % total sugar x 0.01

Oxalate Determination

This was carried out according to (A.O.A.C., 1990). Two grams (2g) of the sample was weighed out and extracted thrice at 50^0C stirred for 1hour with 20ml of 0.3M HCl. The combined extract was diluted to 100ml with distilled water and used for total oxalate estimation. The oxalate was estimated by pipetting about 5ml of the extract which was made alkaline with 1ml of 5M ammonium hydroxide. About 3 drops of phenolphthalein were added to the extract and acetic acid was added in drops. Also about 1ml of 5% CaCl (aq) was then added to the mixture and allowed to stand for 2hours after which it was centrifuged at 3,000 rpm for 15min. The supernatants were discarded and the precipitates washed three times with hot water, thoroughly mixed and centrifuged each time. In the test tube, 2ml of 3M H_2S0_4 was added and the precipitate was dissolved by warming in water bath at 75^0C. The content of the test tube was then titrated with freshly prepared 0.01M $KMnO_4$ at room temperature until the first pink colour appeared throughout the solution. This was then warmed at 75^0C and the titration continued until the pink colour persisted.

$$\% \text{ Oxalate} = Vt/Ws \times Vme \times \text{Titre}$$

Where, Vt = Total volume of titrate = 100
Ws = Weight of the sample = 2g
Vme = Volume – mass equivalent (i.e.1cm^3 of 0.05M $KMnO_4$ is equivalent to 0.00225g anhydrous oxalic acid)

Phytate Determination

This was carried out according to A.O.A.C. (1990). Two (2.0g) of the sample was weighed into a test tube. About 10ml of distilled water was added. The sample was extracted using 2ml of 0.2M HCl (aq). About 0.5ml of the extract was pipetted into a test tube fitted with glass stopper. Then, 1ml of the solution was added in the tube and covered with stopper. The tube was heated in a boiling water bath for 30 min and the tube was covered very well with the stopper for the first 15min. Then the test tube containing the solution was cooled in ice water for 15min and allowed to adjust to room temperature. Then the content of the test tube was mixed very well and centrifuged for 30 min. About 1ml of the supernatant was transferred into another test tube and about 1.5ml of the solution was added. The absorbance at 420nm against distilled water was measured.

$$\% \text{ Phytate} = \frac{Au}{As} \times \frac{C}{W} \times \frac{100}{VA} \times Vf$$

Au = absorbance of test sample
As = Absorbance of standard solution
C = concentration of standard solution
W = Weight of sample used
Vf = Total volume of extract
Va = Volume of extract

Results and Discussion

Effect of Malting on the Proximate Composition of African Yam Bean:

The results of the proximate composition of the samples are shown in Table 1.

Table 1: Mean values of the triplicate determination of the proximate composition of African yam bean

	Soaking time (h)	Germinat-ion time(h)	Ash content (%)	Fat content (%)	Moisture content (%)	Crude fibre (%)	Crude protein (%)	Carbohy-drate (%)
	24 hours	R.S	3.23^{a}	1.64^{a}	9.43^{a}	5.31^{a}	23.24^{a}	58.05^{a}
	24 hours	3.23^{a}	1.63^{a}	9.43^{a}	5.31^{a}	22.63^{b}	58.04^{a}	
	48 hours	3.21^{a}	1.61^{a}	9.37^{a}	5.26^{b}	22.50^{b}	57.84^{a}	
	72 hours	3.20^{a}	1.61^{a}	8.67^{b}	5.21^{c}	3.81^{d}	57.53^{a}	
	96 hours	3.19^{a}	1.59^{a}	8.64^{b}	5.12^{d}	23.81^{d}	57.53^{a}	
$LSD_{0.05}$		0.0264	0.0597	0.3536	0.0540	0.3207	0.5742	
48 hours	24 hours	2.93^{a}	1.61^{a}	9.41^{a}	5.17^{a}	22.63^{a}	58.25^{a}	
	48 hours	2.93^{a}	1.61a	9.30^{a}	5.12^{a}	22.88^{a}	58.20^{a}	
	72 hours	2.91^{a}	1.59^{a}	9.16^{a}	5.06^{a}	23.00^{a}	58.20^{a}	
	96 hours	2.91^{a}	1.57^{a}	8.85^{a}	4.93^{a}	24.38a	57.02^{b}	
$LSD_{0.05}$		0.0251	0.0320	0.3463	0.2235	0.3812	0.9786	

Note: Means with different superscripts along the column have significant difference at $p < 0.05$

R.S = raw sample

Crude Protein Content

From the values obtained from the proximate composition in table 1, the unmalted sample of African yam bean had a crude protein content of 23.24%. This corresponds with the range of protein content in the seeds (15.8% to 34.7%) as reported by (Edem *et al.*, 1990). After malting, the crude protein content of African yam bean soaked for 24h increased from the original 23.24% to 23.8%, with the 24h germinated sample having the least value 22.63% and increased as the time of germination increased with the 96h germinated sample having the highest value 23.81%.For the 48h soaked sample the increase continued as germination increased with the least value for the 24h germinated sample as 22.63% and 96 hours germinated seeds having the highest value at 24.39%. The result

corresponded with earlier reports of increase in protein content during germination of various cereals, legumes and other seeds (Yagoub *et al.*, 2008). This increase could be attributed to a net synthesis of enzyme protein (e.g. protease) by germinating the seed.

Crude Fat Content

The value of the fat content obtained from African yam bean for the unmalted sample was 1.64%. The 24h soaked sample and malted for 24h had the highest value of 1.63%, with the 96h malted sample having the lowest value of 1.59%. There was no significant difference ($p>0.05$) between the successive increases in the time of germination. The 48h soaked and 24h germination had a value of 1.61% which was the highest value of fat content obtained while 96h germination had the lowest value of 1.59%. There was no significant difference ($p> 0.05$) between the 24h and 48h soaked seeds. The observed decrease in the fat contents of the germinated seeds might be due to the increased activities of the lipolytic enzymes during germination. They hydrolyze fats to simpler products which can be used as a source of energy for the developing embryo. Similar observation was made for bambara groundnuts as reported by Elegbede (1998). This decreased fat content implies an increased shelf-life for the germinated seeds compared to the ungerminated ones.

Ash Content

The ash content of the unmalted raw sample had a value of 3.23% while there was no significant difference ($p>0.05$) between the unmalted sample and all the malted samples. The 24h soaked and 24h germinated sample had the highest ash content while the 96h germinated sample had the least ash content. For the 48 hours soaked sample, there was no significant difference between the malted samples with the highest value for the 24h germination (2.93%) and the least value for the 96h germinated seeds 2.91%. These results are in agreement with those reported earlier by several workers (Gopolan *et al.*, 1989; Venderstoep, 1981). Leaching out of solid matter during pre germination, soaking process could be the reason for significant reduction of mineral matter on germination.

Moisture Content

For 24h soaked sample, there was a significant difference ($P < 0.05$) between the raw sample and the malted sample for 72h and 96h. The raw sample had a value of 9.43%. The highest moisture content was for 24h soaked and 24h germinated sample and the least moisture content was for 96h germination. For 48h soaked sample, the 24h germination also had the highest value 9.41% with 96h germination having the lowest value 8.85%. This could be attributed to the results reported by Ohtsubo *et al.* (2005) in which brown rice contained lower moisture in germinated rice samples. As germination proceeded, legumes took up water from the surrounding in order for the metabolic process to commence, also this moisture is equally removed by the application of heat to dry back to its initial moisture content to enable a stable shelf life of the legume.

Crude Fibre Content

The unmalted raw sample had a crude fibre content of 5.31%. There was no significant difference between ($P > 0.05$) the unmalted sample and the 24h soaked and 24h germinated but there was a significant difference ($P < 0.05$) between the raw sample and the remaining germination times (i.e. 48h, 72h, and 96h). The 48h soaked and 24h germinated sample had the highest value 5.17% with the 96h germinated seeds having the least value 4.93%. On germination, soluble and total fibre fractions increased and insoluble fibre fractions reduced significantly ($P < 0.05$). In this study, there were marked reductions of all fibre fractions on dehulling of all legume samples studied. These data agreed with the findings of Ramulu and Udayasekhara (1997) for dehulled green grain, pigeon pea, lentil and chick pea.

Carbohydrate content

There was no significant difference ($p >0.05$) between the unmalted raw sample which had a value of 58.05% and the malted samples. The 24 hours soaked sample and 24 hours germinated sample had the highest value 58.04% while the 96 hours germinated seeds had the least value of 57.75%. Also for the 48 hours soaked samples, the 24 hours germinated sample had the highest value of 58.25% while the 76 hours germinated sample had the lowest value of 57.02%.

The decrease in the total carbohydrate content corroborated the observation of a decrease in carbohydrate content after germination by Yagoub *et al.*, (2008). The decreased carbohydrate levels of the germinated seeds 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.

Effect of Malting on the Anti-Nutritional Properties of African Yam Bean:

The results of the anti-nutritional properties of the samples are shown in Table 2.

Table 2: Mean values of triplicate determination of the anti nutritional composition of african yam bean

Soaking time(h)	Germination time(h)	Phytate (%)	Alkaloid (%)	Saponin (%)	Stachyose (%)	Raffinose (%)	Haemagl-utinin (HUI/g)	Oxalate (%)	Tannin (%)	Polyphenol (%)	HCN Mg/kg	Trypsin inhibitor TUI/g
RS		0.363^{a}	0.43^{a}	0.61^{a}	0.309^{a}	0.077^{a}	138.73^{a}	0.229^{a}	0.230^{a}	0.113^{a}	22.66^{a}	22.09^{a}
	24	0.361^{a}	0.41^{b}	0.57^{b}	0.300^{b}	0.075^{a}	131.53^{b}	0.232^{a}	0.227 a	0.112^{a}	22.62^{a}	22.07^{a}
24 hours	48	0.355^{b}	0.39^{c}	0.53^{c}	0.297^{b}	0.074^{a}	98.90_{c}	0.221^{a}	0.212^{b}	0.112^{a}	17.81^{b}	18.69^{b}
	72	0.351^{b}	0.39^{c}	0.51^{c}	0.270^{c}	0.070^{b}	73.40^{d}	0.214^{a}	0.165^{c}	0.112^{a}	12.15^{c}	11.70^{c}
	96	0.346^{b}	0.37^{d}	0.49^{c}	0.267^{c}	0.067^{c}	58.70^{e}	0.207^{a}	0.121^{d}	0.111^{a}	10.31^{d}	11.64^{d}
$LSD_{0.05}$		0.0058	0.0093	0.0386	0.0058	0.0026	0.3606	0.0136	0.0063	0.0021	0.1207	0.0251
	24	0.350^{a}	0.41^{a}	0.49^{a}	0.275^{a}	0.069^{a}	93.83^{a}	0.229^{a}	0.208^{a}	0.112^{a}	19.88^{a}	18.27^{a}
48 hours	48	0.344^{b}	0.39^{a}	0.43^{b}	0.274^{a}	0.068^{a}	71.70^{b}	0.218^{a}	0.192^{a}	0.111^{a}	11.79^{a}	12.36^{b}
	72	0.341^{b}	0.37^{a}	0.40^{b}	0.253^{b}	0.063^{b}	56.57^{c}	0.207^{a}	0.144^{c}	0.080^{a}	9.70^{c}	9.48^{c}
	96	0.339^{a}	0.35^{a}	0.39^{b}	0.235^{c}	0.059^{c}	33.53^{d}	0.199^{a}	0.098^{d}	0.080^{a}	9.09^{d}	8.70^{d}
$LSD_{0.05}$		0.0058	0.0527	0.0340	0.0018	0.0036	0.1878	0.0115	0.0030	0.0617	0.0015	0.0237

Note: Means with different superscripts along the column have significant difference at $p< 0.05$
R.S = raw sample

Phytate
The phytic acid of the unmalted sample had a value of 0.363%. There was a significant difference ($P < 0.05$) between the malted samples and the unmalted sample. The 24h soaked and 24h germinated samples had the highest level of phytic acid while the increase in germination time further decreased the phytic acid in the sample. The 48 hours soaked and 24 hours germinated sample had a value of 0.350% while 96 hours germination had the least content of phytic acid 0.339%. The decrease in phytic acid in germinated samples were comparable to the results reported for other germinated legumes including African oil bean (Enujiugha *et al.,* 2003) and pearl millet (Kumar and Chauhan,1993). Decrease in phytic acid content during germination could be due to increase in phytase activity as reported by Eskin and Wiebe (1983) in faba beans.

Tannin
The unmalted raw sample was significantly different ($p<0.05$) from the malted sample. The raw sample had 0.230% of tannin. The 24 hours soaked and 24 hours germinated sample had a value of 0.227% and the 24 hours soaking and 96 hours germinated sample had the least value 0.121%. This result implies that there was a significant reduction in tannin as the time of germination increased. This could be as a result of the leaching of soluble tannin compounds during soaking and was further reduced during germination. Also, the 48 hours soaked and 24 hours germination had the highest value of 0.208% while the 96 hours germinated sample had the least value of 0.098%.

Saponin
The unmalted raw seeds were significantly different ($p<0.05$) from the malted samples. The raw sample had a value of 0.61%. The 24 hours soaked sample and 24 hours malted sample had the highest value of 0.57% while the 96 hours malted sample had the least value 0.49%. The 48 hours soaked and 24 hours malted sample had the highest (0.49%) while the 96 hours germinated sample had the lowest value (0.39%). This implies that malting reduces a large portion of saponin although not all was removed. Since the intake of this toxin causes a reduction in the uptake of certain nutrients including glucose and cholesterol at the gut through intralumenal physiochemical interaction (Price *et al.,* 1987), the malting of the African yam bean causes a reduction of this adverse effect.

Trypsin Inhibitor
The result showed a significant difference ($P < 0.05$) between the raw sample and the malted samples. The 24 hours soaked and the 24 hours malted sample had the highest value of 22.07 TUI/g while the 96 hours malted sample had the lowest value 11.64 TUI/g. The 48 hours soaked sample and the 24 hours malted sample had the highest value 18.27 TUI/g while he 96 hours malted sample had the lowest value 8.70TUI/g. These seemingly suggest that increasing the germination time may ensure a reasonable reduction or elimination of trypsin inhibitor in African Yam bean flour.

Haemagglutinin
There was a significant difference ($p<0.05$) between the raw sample and the malted samples. The 24 hours soaked sample and the 24 hours malted sample showed the highest value of 131.53 HUI/g and the lowest value was that of 96 hours malted sample 58.70HUI/g. The 48 hours soaked sample and the 24 hours malted sample had the highest value of 93.83 HUI/g while the 96 hours malted sample had the lowest value of 33.53 HUI/g. This result suggests that there would be a great reduction in the ability of the sugar binding proteins to bind and agglutinate red blood cells (Liener, 1985) because of the germination process that took place in the seeds.

Oligosaccharides
For stachyose, there was a significant difference ($p < 0.05$) between the raw sample and the malted samples, with the 24 hours soaked sample and 24 hours malted sample having the highest value 0.309% while the 96 hours malted sample had the least value of 0.267%. For the 48 hours soaked sample and the 24 hours malted sample had the highest value of 0.275% and the 96 hours malted sample having the least value of 0.235%.
For raffinose, the raw sample had a value of 0.077% which is significantly different ($P < 0.05$) from the malted sample with the 24 hours soaked sample and the 24 hours malted sample having the highest value of 0.075% and the 96 hours malted sample had the least value 0.067%. The 48 hours soaked sample and 24 hours malted sample had the highest value of 0.069% while the 96 hours malted sample had the least value of 0.059%. The result suggests that malting must have caused a significant reduction of the flatulence-producing sugars by hydrolysis of the complex sugars to simple disaccharides and monosaccharides to levels that would reduce the cause of flatulence in the body. Dehulling also helped to decrease them (Nwosu, 2010).

Hydrogen Cyanide
There was a significant difference ($p < 0.05$) between the unmalted raw sample and the malted sample. The 24 hours soaked and 24 hours malted sample had the highest value of 22.62mg/kg while that of 96 hours malted sample had the least value of 10.31mg/kg. The 48 hours soaked and 24 hours malted sample had the highest value of 19.88mg/kg while that of 96 hours malted sample had the least value of 9.09mg/kg. The reduction in the values gotten was as a result of its reduction during malting. Due to this reduction, the

ability to cause adverse effect in the system like gastrointestinal inflammation and inhibition of cellular respiration is greatly avoided or eliminated.

Oxalate

The study showed that there was no significant difference ($P > 0.05$) between the raw sample and the malted samples. The values range between 0.229% - 0.207%. The 24 hours soaked sample and the 24 hours germinated sample, had the highest value of 0.232% and that of 96 hours germinated sample had the least value of 0.20%. For 48 hours soaked sample, the 24 hours germinated sample had the highest value which was 0.229% whereas the 96 hours germinated sample contained the least value 0.199%. The oxalate value was lower when compared with the earlier reports of Umoren *et al*. (2005). The amount of oxalate ingested may be an important risk factor in the development of idiopathic calcium oxalate nephrolithiasis (Holmes and Kennedy, 2000) which is eliminated by the malting process.

Alkaloids

The result showed that there is a significant difference between the malted sample and the unmalted sample. The value ranged between 0.43% - 0.37%. The 24 hours soaked sample and 24 hours malted sample had the highest value of 0.41% while the 96 hours malted sample had the least value of 0.37%. The 48 hours soaked sample had a range between 0.41% - 0.35%, with the 24 hours malted sample having the highest value while the 96 hours malted sample having the least value of 0.35%. Due to the reduction in the alkaloid content in this study, there would be an elimination of neurological disorder and haemolysis in the body system (Saito *et al.*, 1990) when AYB is consumed.

Phenols

There was no significant difference ($p > 0.05$) between the raw unmalted sample and the malted samples. The range was between 0.113 - 0.111%. The 24 hours soaked sample and 24 hours malted sample had the highest value of 0.112% while the 96 hours malted sample had the least value in the range. Also, for the 48 hours soaked and 24 hours malted had the highest value in the range of 0.112%-0.08% while the 96 hours malted sample had the least value. The result revealed that there was a significant reduction in the phenolic compounds in which the sprouting treatments were very effective in reducing total phenols. The reduction might also be due to leaching and an increased enzymatic hydrolysis which might have facilitated the reduction of total phenols in the sprouting treatments (Bishnoi *et al*., 1994).

Effect of Malting on the Dehulling of African Yam Bean.

Malting enabled the ease of dehulling of the seeds by loosening the hulls from the cotyledon during the soaking of the seeds which caused an increase in protein content and caused a reduction in tannin and polyphenol content of the malted seeds (Alonso *et al.*, 2000).

Conclusion

In this study, the effect of malting on the proximate composition of African yam bean showed that it has a high nutritional value compared with other legumes like soybean, cowpea, groundnut, thus it could be used as a protein supplement. The malting process could also lead to a shortened cooking time of the African yam bean. There was a significant variation with respect to anti-nutritional factors wherein sprouting/germinating treatments reduced the anti-nutritional factors while increasing the enzyme activities and conserving mineral content of the legume. The process of soaking and malting helped to release the hulls from the cotyledon which helped in the reduction of major anti-nutritional factors in African yam bean. Germination and dehulling processes improve the quality of legumes by enhancing the bioavailability and digestibility of nutrients and reducing anti-nutrients.

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