

INFLUENCE OF SOAKING AND HYDROTHERMAL TECHNIQUES ON ANTINUTRITIONAL COMPONENTS AND *IN VITRO* MULTIENZYMES PROTEIN DIGESTIBILITY OF V*IGNA RACEMOSA* – AN UNDERUTILISED HARD-TO-COOK LEGUME

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Abstract

This study is aimed at determining the effects of simple and cheap hydrothermal techniques on the antinutrtional components and in vitro multienzymes protein digestibility of Vigna racemosa, an underutilised hard-to-cook legume. Seeds of V. racemosa were subjected to aqueous soaking and four hydrothermal processing methods — atmospheric boiling and steaming; pressure boiling and steaming. The effects of soaking and the hydrothermal techniques on the antinutrtional components (phytic acid, saponin, tannin and trypsin inhibitor activity) and the in vitro protein digestibility were investigated. Increase in hydration level caused more seepage of the antinutrients into the soaking water. With the exception of trypsin inhibitor activity that was totally eliminated, other antinutritonal components experienced significant reduction (p<0.05) when the seeds were subjected to the various hydrothermal processing techniques. The raw sample has protein digestibility of 40.11% while steaming at atmospheric pressure and steaming at elevated pressure caused percentage digestibility of 87.11% and 87.36%, respectively. The boiling treatments resulted in better percentage protein digestibility of 87.91%. Application of these simple and cost effective hydrothermal processing methods resulted in significant decrease in the antinutritional components and hence improvement of protein digestibility. Increase in utilisation of this legume for foods could strengthen dietary diversity and healthy eating habit and thus alleviate the problems of protein energy malnutrition and food insecurity.

Keywords: Hydrothermal techniques, underutilised legume, digestibility, *Vigna racemosa*, antinutritional components.

Received: 26.05.2017 Received in revised form: 19.06.2017 Accepted: 18.07.2017

1. INTRODUCTION

The nutritional importance of legumes as sources of protein and carbohydrates in human nutrition cannot be over emphasized in many nations of the world and especially in developing countries where large segments of the population suffer from protein energy malnutrition (PEM). One of the most serious challenges facing the increasing population in most developing countries is the scarcity of food for human population and feed for livestock industries. Although the use of conventional legumes for foods and feeds is not uncommon in many countries, their production is not enough to meet the requirements of the increasing population

(Siddhuraju and Becker, 2001). Moreover, much emphasis has been placed by food scientist, nutritionist and health professionals on the need for increased consumption of protein foods. This is because protein is an important nutrients necessary for building, repair and maintenance of body tissues (Sunetra, 2009). Due to high cost of protein foods, deficiency of protein in the form of protein energy malnutrition is still common in many parts of the world especially in developing countries. Conventional legumes could not meet the need of a large segment of the world population because of the low level of production. Moreover, over dependence on common legumes has resulted in hike in price. Hence, there is need for exploitation of hitherto



neglected legumes (Agbede and Aletor, 2005). Legumes are a useful components of balanced diets. Legumes reduce the incidences of cardiovascular diseases (CVD), diabetes and cancer (Polhill, 1994). They have low allergenic capacity compared with some other sources of protein (Graham and Vance, 2003). Legumes have many uses in biodegradable plastics, dyes and biodiesel (Gept et al., 2005). Although the problems of malnutrition and hunger can be alleviated through the use of a group of plants such as the presence of legumes, antinutrients constitutes a great limitation to their utilisation. antinutrients These include tannin, heamaglutinin, cyanide, saponin, protease inhibitor, oligosaccharades etc.

Vigna racemosa is a lesser known legume in South West Nigeria. It is not available in much commercial quantity but it is planted by peasant farmers for subsistence purpose. It is often consumed as a backup food when the stocks of other foodstuffs are being depleted. Information on the nutrients antinutrients components of V. racemosa has been reported in an earlier study (Ojo et al., 2014). However, information on the effect of soaking and hydrothermal techniques on the antinutritional components and improvement of the in vitro protein digestibility of V. racemosa is not available. Therefore, this study seeks to determine the effects of simple and cheap processing techniques on the antinutrtional components and in vitro multienzymes protein digestibility of V. racemosa. It is hoped that provision of such information will enhance further utilisation and thus solve the problem of hunger and PEM in many developing countries of the world.

2. MATERIAL AND METHODS

MATERIAL

The seeds of *Vigna racemosa* locally called *Gbomagungi* (Figure 1), were obtained from a

local market in Ago-Are (8.670 N, 3.400 E), Oyo State, Nigeria. The seeds were dry-cleaned thoroughly and the immature seeds and extraneous particles were removed. The cleaned seeds were stored in a plastic container at room temperature prior to further processing and analysis.

Legume seed processing

Whole seeds of the legume were subjected to the following processing methods: soaking, atmospheric boiling, boiling at high pressure cooker, atmospheric steaming and steaming at high steam pressure.

Hydration of the legume seeds

The legume seeds were hydrated by soaking about 500 g of the cleaned seeds in 2.5 L of distilled water in a beaker for 24 hours at ambient (25±3 °C) During soaking, the water absorption of the seeds was measured every sixty minutes for the first six hours and thereafter measured every two hours. Water was removed from the soaked seeds at the time appointed by mopping gently with a wollen towel, weighed and then returned into the soaking water. After soaking, the moisture content of the seeds was calculated. Moreover, the variation of the change in moisture with time was presented by plotting the water absorption curve. The duration of soaking of the seeds with desired hydration level was using polynomial estimated equation respective absorption water curves. Subsequently, the seeds were boiled or steamed using the methods described hereby:

Atmospheric boiling (B1)

Using a domestic cooker, about 500 g of previously hydrated seeds were boiled in distilled water at normal atmospheric pressure. Cooking time was determined using tactile method (Vindiolla *et al.*, 1996; Ojo *et al.*, 2016). The boiled legume seeds were allowed to cool to room temperature and poured unto a plastic tray. The cooled seeds were dried in a cabinet dryer at 45±5 °C. After drying, the



sample was stored in a polyethylene bag before further analysis.

Pressure boiling (B2)

Boiling of previously hydrated seeds was carried out at 80±8 KPa using a pressure cooker ---Binatone Model-PC-5001. About 500 g of the legume seeds was poured into a glass flask containing 2.5 L of distilled water. Covering the flask with aluminium foil, it was placed on a hot plate to boil. The flask containing the seeds was then placed in the preheated pressure cooker containing 2.5 L of boiling water. Counting of cooking time commenced when steam started oozing out of pressure cork. Cooking time determined using tactile method (Vindiolla et al., 1996; Ojo et al., 2016). After boiling, the pressure was released, and the seeds were cooled to ambient (25±3 °C) and dried in a cabinet dryer at 45±5 °C. After drying, the sample was store in a polyethylene bag.

Atmospheric steaming (S1)

A domestic cooker was used to steam the presoaked legume seeds at atmospheric pressure. About 500 g of the legume seeds were put on a stainless steel tray in the steam cooker containing 2.5 L of water. The lid was securely tightened and the steaming was carried out. Cooking time was determined using tactile method (Vindiolla *et al.*, 1996; Ojo *et al.*, 2016). After steaming, the seeds were cooled and dried at 45±5 °C. The dried seeds were stored in a polyethylene bag.



Fig. 1: Vigna racemosa (Gbomagungi)

Pressure steaming (S2)

Steaming of the legume seeds was carried out at 80 ± 8 KPa using a pressure cooker --- Binatone Model-PC-5001. The legume seeds (500 g) were steamed on a stainless steel tray in the pressure cooker at the elevated pressure. The sample was then cooled to room temperature in a plastic container and dried at 45 ± 5 °C. After drying the sample was kept in a polyethylene bag before further analysis.

Trypsin inhibitor activity (TIA) determination

The trypsin inhibitor activity was determined using the procedure of Smith *et al.* (2000). Benzoyl-DL-arginine-P-

nitroaulidehydrochloric (BAPNA) manufactured by Zefa Laboratory Service, Germany was used as substrate. Crystalline porcine pancreatic trypsin (trypsin ZF 93615.0025) 40 mg (Boehinge Bellane loives) manufactured by Zefa Laboratory Service, Germany and dissolved in 0.001M HCl such that standard trypsin solution contained 40 µg trypsin.

Using n-hexane, 1 g of the milled legume sample was deffatted for about three hours. About 50 cm³ of 0.01M NaOH was added to the sample. The pH of the resultant mixture was adjusted to 9.5 with solution of 0.1M NaOH. The mixture was then grinded in a blender for two minutes before it was centrifuged at 100 rpm for 10 minutes. Distilled water was used to dilute the sample so as to obtain a dilution in which 1 cm³ of the extract produced trypsin inhibition activity of between 40 and 60%. The BAPNA substrate as well as the trypsin solutions were used with the sample dilution at 37 °C as reported by Kakade et al. (2009). The mixture was allowed to react at 37 °C in a water bath (Uniscope model SM 902B). The absorbance of the resulting solution was read at 410 nm against blank. sample

Trypsin inhibitor was calculated as



 $TIA = [2.632 \times DF \times A]/W = mg$ pure tryp sin/ g sample

where, DF = dilution factor; A = change in absorbance (pure trypsin and sample extract);

W = weight of the sample (g)

Determination of tannin content

The tannin content of the legume seed was determined by modifying the procedure of Makker (1994). The seed flour was defatted using diethyl ether, ground and sieved through 500 µm sieve. About 0.2 mg of the defatted flour was extracted with 10 cm³ of 70% aqueous acetone for 2 hours in a water bath (Uniscope model SM 902B) at 30 °C (Aletor, 1993). The extract was centrifuged at 3500 rpm for 20 minutes and 0.05 cm³ of the supernatant was used. Increasing concentration of standard tannic acid was prepared and 0.5 cm³ folin-Ciocalteu reagent was added and their absorbance measured at 725 nm against distilled water using a spectrophotometer (Model - Buck 205). The absorbance of the various tannic acid concentrations was used to obtain a regression equation that was used to determine tannic acid in each sample extract. regression equation The was Y = 0.021X - 0.01

[Y = absorbance; $X = tannic \ acid \ (\mu g)$] Tannic acid from each sample was determined and expressed as mg/g of the flour sample.

Determination of total saponin content (TSC)

The procedure of Makker and Becker (1997) was modified for use. About 0.5 g of the dried, grinded legume sample was defatted with 10 cm3 of petroleum ether by shaking for 4 hours and then the residue was extracted twice with 5 cm³ of aqueous methanol on an orbit shaker by shaking for 4 hours each. The extract was stored at 40 °C in the dark for use.

TSC was determined using spectrophotometric method (Hai *et al.*, 1996). About 0.1 cm³ of the legume extract, 0.4 cm³ of 80% methanol solution, 0.5 cm³ of freshly prepared vanillin solution (in ethanol) and 50 cm³ of 72%

sulphuric acid were mixed together thoroughly in an ice water bath. The mixture was warmed in a water bath at 60 °C for ten minutes and then cooled in ice cold water. Absorbance at 544 nm was recorded against the reagents blank with a UV-visible spectrophpotometer (UV 160 Shimadzu). The results were expressed as mg of sova saponin equivalent/g of legume on a dried weight basis from a standard curve of different concentration of crude soya saponin (contained a minimum of 80% saponin, Sigma- Aldrich) in aqueous methanol 2009). (Xu and Chang,

Determination of phytic acid

Phytic acid in the legume sample was extracted according to the method of Gao et al. (2007). About 0.5 g of the raw dried sample deffated with 10 cm³ of petroleum ether by shaking for 4 hours and then the residue was extracted with 10 cm³ of 24% HCl by shaking on the orbit shaker for 6 hours. The extract was stored at 4 °C in the dark prior to further analysis. Phytic acid was determined using the colourimetric method described by Gao et al. (2007) with slight modification. About 0.1 cm³ of the extract was diluted by 29 cm3 of distilled water, and then 3 cm³ of this diluted sample was combined with 1 cm³ of freshly prepared Wadde reagent (0.003% FeCl₃.6H₂O + Sulfosalicylic acid) in a 15 cm³ tube. The were thoroughly contents mixed centrifuged at 5500 rpm at 10 °C for 10 minutes. Calibration standards of different concentrations of 0, 5, 10, 20, 25, 75 or 100 mg/cm³ of phytic acid were prepared by diluting 10 mg/cm³ of phytic acid stock water. solution with distilled Using UV Spectrophotometer (UV160 Shimadzu), the absorbance of the sample was read at 500 nm against water as blank. Results obtained were reported on dry weight basis as milligrams of phytic acid per gram of legume sample (mg/g).

Determination of protein digestibility

The in vitro protein digestibility of the legume



seeds was determined (Hsu et al., 1997). The enzymes used were porcine pancreatic trypsin bovine pancreatic (Z.F)93615.0025). chymotrypsin (Z.F 27270) and porcine (Z.F 77163.0500) intestinal peptidase manufactured by Zefa Laboratory Service, Germany. The activity of the enzymes was initially determined before use by using them to digest casein. Appropriate grammes of the flour was dissolved in 50 cm³ distilled water to sample suspension of 6.25 give protein/cm³. The sample suspension adjusted to pH 8 and incubated in water bath at 37 °C with constant stirring. Fresh multienzyme solution was prepared to contain 1.6 mg trypsin, 3.1 mg chymotrypsin and 1.4 mg peptide dissolved in 1 cm3 distilled water. The pH of enzyme solution was maintained at 8 cm3 of multi-enzyme solution was added to each sample suspension with constant stirring at 37 °C. After adding the enzyme solution, the pH of each sample was determined after 10 minutes and 15 minutes, respectively. The in vitro multienzymes protein digestibility was then determined with the following equation.

A= 210.464 - 18103B

(A= percentage protein digestibility; B= pH of sample suspension)

Statistical analysis

Statistical analyses of all the data obtained in three replicates were carried out with Statistical Analysis Software (SAS, version 15.0, 2005). Analysis of variance (ANOVA) was used to determine the significance difference (p<0.05) and the means were separated using Duncan's test.

3. RESULTS AND DISCUSSION

Antinutritional components of *V. racemosa* **at varying hydration levels** Figure 2 shows the water absorption curve for *Vigna racemosa* while Table 1 shows the effect of soaking at varying hydration levels on the concentration of antinutritional components. In general, soaking reduced the concentration of

the antinutrients in the legume seeds. The percentage reduction differed at varying hydration levels. The raw seeds of V. racemosa contained 63.15 mg/g of phytic acid. After soaking, the percentage reduction ranged from 0.21% at 10% hydration level to 2.75% at 100% hydration level. The percentage reduction of phytic of acid obtained in this study was relatively lower than 37% reported for seeds of B. purpurea soaked in distilled water at 24 °C for 6 hours (Vijayakumani et al., 2007). In another study, Udensi et al. (2008) reported percentage reduction of 36.0% of phytic acid after soaking for 24 hours at ambient temperature. Reduction of phytic acid during soaking could be attributed to leaching. Loss of phytic acid can also be due to degradation of phytate molecule followed by diffusion of phytase enzymes which activated in the seed (Siddhuraju et al., 2001). Saponin content of the legume seeds reduced at varying hydration levels. At hydration levels of 50%, 75% and 100%, the percentage reduction of 2.33, 2.92 and 3.11%, respectively were observed. There was a progressive decrease in the saponin content of the legume seed as the hydration increased. The concentration of tannin before soaking was 28.97 mg/g. After soaking the percentage reduction range was 3.21% to 15.53%. Vijayakumani *et al.* (2007) reported reduction in tannin content of some beans after soaking in different solutions. Reduction in tannin during soaking could be due to leaching out of the polyphenols into the soaking water. Tannin are polyphenols which are soluble water soluble in nature and are mostly located in the seed coat.

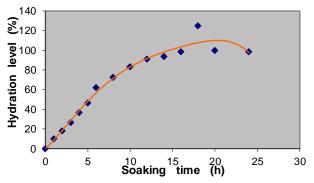


Fig. 2: Water absorption curve for Vigna racemosa



TIA was reduced at varying levels of hydration. Similar change in chemical composition was reported when *Glycine max* was processed by soaking for 24 hours at ambient temperature (Okorie, *et al.*, 2004).

Effect of hydrothermal techniques on the antinutritional components of *V. racemosa*. As presented in Table 2, all the hydrothermal processing techniques employed had reduction effect on the antinutrional components of *V. racemopsa*. The percentage reduction was dependent on the the methods of processing. The concentration of phytic acid in the raw

seed was 63.15 mg/g. Boiling at normal atmospheric pressure reduced the phytic acid by 65.87% while steaming at atmospheric pressure reduced the phytic acid content by 59.03%. Boiling at elevated pressure led to the percentage reduction of 62.41%. The results were comparable to but higher than the value obtained by Wang *et al.* (2009) who reported a loss of 15% reduction in phytate level after cooking. Also, seeds of *B. pupurea* lost 29% phytic acid during cooking (Vijayakumani *et al.*, 2007).

Table 1: Concentration of antinutrtional components (mg/g) in *Vigna racemosa* before and after soaking at varying hydration levels (mg/g)

A			Hydration	Hydration level (%)		
Antinutrtional components	0	10	25	50	75	100
Phytic acid	$63.15d \pm 0.28$	$63.02c \pm 0.04$ $\{0.21\}$	62.71b ± 0.30 {0.69}	$61.42a \pm 0.21$ $\{2.74\}$	61.41a ± 0.3±0 {2.76}	61.41a ± 0.08 {2.75}
Saponin	5.15d ±0.48	$5.08c \pm 0.31$ {1.17}	5.07c ±0. 40 {1.36}	5.02b± 0.21 {2.33}	4.99a ±0. 17 {2.92}	$4.98a \pm 0.10$ {3.11}
Trypsin inhibitor	$9.72f \pm 0.04$	$9.54e \pm 0.01$ {1.85}	$9.41d \pm 0.03$ {3.19}	$9.08c \pm 0.01$ {6.58}	$8.68b \pm 0.11 \\ \{10.69\}$	$8.58a \pm 0.20$ {11.73}
Tannin	$28.97e \pm 0.32$	$28.04d \pm 0.10$ {3.21	$27.16c \pm 0.02 $ {6.25}	$26.55b \pm 0.13$ $\{8.35\}$	$24.47a \pm 0.24 \\ \{15.53\}$	$24.47a \pm 0.24$ {15.53}

Means with different letters along the same row are different significantly (p<0.05). Percentage loss is presented in parenthesis.

Table 2: Antinutritional components of Vigna racemosa as influenced by hydrothermal processing methods

Antinutrtional components	Processing conditions						
	A	B1	S1	B2	S2		
Phytic acid	63.15d±0.28	21.55a±0.42 {65.87}	25.87c±1.01 {59.03}	23.74b±0.30 {62.41}	25.10c±0.57 {60.25}		
Saponin	5.15e±0.48	1.27a±0.06 {75.34} 0.00±0.00 ^a	1.48c±0.02 {71.26}	1.39b±0.87 {73.01}	1.55d±0.35 {69.90}		
Trypsin inhibitor activity	9.72b±0.04	{100.00}	$0.00\pm0.00^{a}\ \{100.00\}$	0.00±0.00ª {100.00}	$0.00\pm0.00^{a} \ \{100.00\}$		
Tannin	28.97c±0.32	9.02a±0.13 {68.86}	10.15b±0.21 {64.96}	9.38a±0.34 {67.62}	10.13d±0.42 {65.03}		

Means with different letters along the same row are different significantly (p<0.05). Percentage decrease is written in parenthesis. A = raw dried sample; B1= atmospheric boiling; B2 = Pressure boiling; S1 = Atmospheric steaming; S2 = Pressure steaming



Table 3: In vitro multienzyme protein digestibility of Vigna racemosa before and after hydrothermal processing

Processing _	10 min	15 min			
Method	рН	% Digestibility	pН	% Digestibility	
A	9.14	$40.11a \pm 0.09$	9.41	$40.11a \pm 0.17$	
B1	6.80	$87.36c \pm 0.08 \\ \{117.80\}$	6.77	$87.91c \pm 0.06 \\ \{119.17\}$	
S1	6.82	87.00b ± 0.06 {116.90}	6.80	$87.18b \pm 0.24$ {117.35}	
B2	6.80	87.36c ± 0.14 {117.80}	6.77	$87.91c \pm 0.07 \\ \{119.17\}$	
S2	6.82	87.00b ± 0.06 {116.90}	6.80	$87.36b \pm 0.04 \\ \{117.80\}$	

Means with different letters along the same column are significantly different (p<0.05). Percentage increase in protein digestibility is written in parenthesis.

A = raw dried sample; B1= atmospheric boiling; B2 = Pressure boiling; S1 = Atmospheric steaming; S2 = Pressure steaming

These discrepancies in the percenteage in the reduction may be due differences in the legume samples, their sources and post-harvest handling methods.

Apart from leaching, in decrease the concentration of phytic acid during hydrothermal processing may be partly due to formation of insoluble complexes between phytic acid and other food components such as protein and minerals (Xu and Chang, 2008). Moreover, chemical degradation of phytic acid to the inositol hexaphosphate hydrolysed to penta and tetra phosphate could also lead to loss of phytic acid during hydrothermal processing. Phytic acid, an antinutritional component naturally occurring in legumes is of major concern in nutrition because it chelates mineral cations and interact with proteins forming insoluble complexes.

Formation of these complexes leads to reduced bioavailability of minerals such as calcium, iron and zinc. It also reduces digestibility of protein (Reyden and Selvendran, 1993). However, low levels of phytic acid have been reported to be of health benefits as antioxidants. Phytic acid has been reported to

lower blood glucose response by reducing the rate of starch digestibility and also slow down emptying of the gastric (Seaman *et al.*, 2003; Famularo *et al.*, 2005). Moreover, phytic acid has been observed to have anticancer effects in the colon and mammary gland in rodent models and in various cell lines in vitro (Shamsudin, 2002). Therefore, reduction of phytic acid will enhance bioavailability of proteins and mineral elements in addition to some health benefit effects.

All the hydrothermal techniques had significant effects on the level of saponin in the seed. The raw seeds of V. racemosa had 5.15 mg/g saponin. The percentage reduction in the saponin content was in the range of 69.90 to 75.34% after hydrothermal processing. As in the case of phytic acid, boiling was observed to higher percentage reduction than steaming. In an earlier study, Nwosu (2006) reported 100% reduction in saponin when Bosque amgolensis seed was boiled for 60 minutes. In a dose dependent treatment, soaking followed by irradiation increased the reduction of saponin. At an irradiation dose of 6 KGy, a significant reduction in saponin was observed (Siddhuraju et al., 2002). Loss of



saponin during hydrothermal treatment could be partly due to degradation of 1, 6 glycosidic linkage in the oligosaccharides consequently increase the levels of free sugar (Machaiah et al., 1999). Trypsin inhibitor was present at the concentration of 9.72 mg/g. As presented in Table 2, all the four hydrothermal techniques caused 100% reduction in trypsin inhibitor activity. This results agree with the findings on some cooked common beans where reduction in trypsin activity of between 91.4 to were reported. (Parades-lopez and 99.9% Harry, 2009). Trypsin inhibitor is a protease inhibitor which inhibits proteolytic enzymes hydrolysis. impairing protein thereby Destruction of trypsin inhibitor by thermal treatment might be due to denaturation of protein structure of the trypsin inhibitor (Udensi, et al., 2008)

There was significant change (p<0.05) in the of tannin after hydrothermal processing. A value of 28.97 mg/g of tannin was recorded for the raw seed. Boiling and steaming at elevated pressure resulted in percentage reduction of 67.62 and 65.03%, respectively. The percentage reduction of 68.86 was recorded when the legume seed was boiled at normal atmospheric pressure. Boiling of the seeds of Senna occidentalis resulted in 42.19% reduction in tannin (Abdullahi et al., 2007). However, boiling of Bosquia angolensis seed for 40 minutes was sufficient to eliminate the tannin content of the seed (Nwosu, 2006). Loss of tannin during hydrothermal could be attributed to leaching. Tannins are phenols soluble in water (Siddhuraju et al., 2002). Biological effects of tannin in human and animal species vary considerably. Tannin forms complexes with protein, carbohydrates certain metal ions and other polymers (Bressani et al., 2002). Tannin in the diets of rats, chicks and duckling has been reported to produce reduction in growth rates, protein utilisation and dry matter digestibility (Reddy et al., 1995). Feeding of tannic acid to rats at 5% of the diet resulted lower weight gains but higher levels caused a marked growth depression (Hurrel et al., 2003). Therefore, unprocessed sample cannot be used for animal feed.

In vitro multienzymes protein digestibility of the legume seeds (IVPD) The IVPD of the legume seeds before and after application of hydrothermal techniques are presented in Table 3. In previous findings, it has been reported that the bioavailability of a nutrients is not determined by its mere presence in a food (Fagbemi et al., 2007; Ayo et al., 2007; Ojo et al., 2014). Hence the need for in vitro digestibility studies. Each of the processing techniques – B1, B2, S1 and S2 had significant effects (p<0.05) on the percentage digestibility of the legume seeds. The raw seeds have relatively low digestibility of 40.11% at 10 minutes. This is, in all likelihood, due to the presence of naturally occurring inherent anti nutritional components. Low values of *in vitro* protein digestibility of 5.4% for soya bean and 26.2% for African yam bean were reported (Adewusi and Osuntogun, 1991). After hydrothermal treatment which led to the significant reduction of in the concentrations of the antinutrtional components, improvements were observed for the IVPD. The highest IVPD of 87.91% at 15 minutes was recorded when the legume was processed by boiling while steaming at atmospheric pressure resulted in 87.18% protein digestibility. The IVPD after 15 minutes for Artocarpus altilis, Telfaria occidentalis and Anacardicum occidentalis after boiling were 78.02, 84.7 and 82.1%, respectively (Fagbemi et al., 2005). Different processing methods such as germination, fermentation, cooking, baking and defatting have been employed to increase the percentage digestibility in foods (Oyebode et al., 2007). Although heat processing can significantly affect the protein quality of legumes, wet heat processing methods have been reported to improve digestibility of protein more than dry heat methods. This is probably due to the fact that wet heating destroys protease inhibitors

4. CONCLUSION

highly digestible.

Hydrothermal techniques employed--- B1, B2,

and open up the protein structure through

denaturation. The seeds of this legume is



S1 and S2 can effectively reduce and/or remove antinutritional components in Reduction of antinutritional racemosa. components in this legume after hydrothermal processing should improve the nutritive value of the seed and dishes prepared from them. Moreover the hydrothermal techniques can significant increase cause in protein digestibility. Boiling is better than steaming in improving protein digestibility. With high values of percentage protein digestibility after hydrothermal processing, the possibilities of further food utilisation of this underutilised hard-to-cook legume is expedient in local dishes such as akara, ekuru, moimoin and gbegiri. Consumption of such dishes will make cheap and easy-to-digest protein available to many consumers and thus help to alleviate the problems of food insecurity and PEM in developing countries.

5.REFERENCES

- [1] Abdullahi, S. A., Tanko, D. and Bankosi, I. E. (2007). "Determination of the proximate antinutrients and amino composition of raw and boiled *Senna occidentalis* seeds" in Elemo, G. N. (Ed). *Nigerian Institute of Food Science and Technology* 2007 proceedings of the 31st annual conference, Abuja, 156-157.
- [2] Adewusi, S. R. A. and Osuntogun, B. A. (1991). "Effect of cooking on tannin content, trypsin inhibitor activity and *in vitro* digestibility of some legume seeds in Nigeria". *Nigerian Food Journal* 9: 139-153.
- [3] Agbede, O. and Aletor, A. (2005). 'Studies of the chemical composition and protein quality evaluation of differently processed *Canavalia ensiformis* and *Mucuna pruriens* seed flours". *Journal of Food Composition and Analysis* 18; 89–103.
- [4] Aletor, V. A. (1993). "Allelochemical in plant food and feeding stuffs: Nutritional, biochemical and psychopathological aspects in animal production". *Journal of Vetenary and Human Toxicology* 35, pp 57 67.
- [5] A., Ayo, U. A., Nkama, I. and Adewori, R. (2007). "Physiochemical, *In vitro* digestibility and organoleptic evaluation of "*Acha*" wheat biscuit supplemented with *soyabean* flour". *Nigerian Food Journal* 25(1); 77–85.

- [6] Bressani, R.; Elias, E. G. and Braham, E. (2002). "Reduction of Digestibility of legume proteins by tannins". *Journal of Plant Foods* 4; 43-55.
- [7] Fagbemi, T. N. (2007). "Effects of processing on the nutritional composition of fluted pumpkin (*Telfairia occidentalis*) seed flour". *Nigerian Food Journal* 25 (1); 1-22.
- [8] Fagbemi, T. N., Oshodi, A. A. and Ipinmoroti, O. (2005). "Processing effects on some antinutritional factors and *in vitro* multi enzyme protein digestibility (IVPD) of three tropical seeds: Breadnut (*Artocarpus altilis*), Cashew nut (*Anarcardium occidentale*) and fluted pumpkin (*Telfairia occidentalis*)". *Pakistan Journal of Nutrition* 4 (4); 250 –256.
- [9] Famularo, G., De Simone, C., Pamdey, V., Sahu, A. R. and Inisola, G. (2005). "Probiotic lactobacilli: an innovative tool to correct the malabsorption syndrome of vegetarians" Meal Hypotheses 65(6); 1132–1135.
- [10] Gao, Y., Shany, M. A., Mamof, S., Biyashev, R. M., Graham, E. A., Kwanyuen, P., Burton, J. W., Buss, G. R. (2007). "A modified colourimetric method for phytic acid analysis in soyabean". *Crop Science* 47: 1797–1803.
- [11] Gepts, P., Beavis, W. D., Brumones, E. C., Shoemaker, R. C., Stalker, H. J., Weeden, N. F. and Young, N. D. (2005). "Legumes as a nodule plant family. Genomics for food and feed report of the cross legume advances through genomics conference". *Plant Physiology* 137; 1228 1235.
- [12] Graham, P. H. and Vance, C. P. (2003). "Legumes: Importance and constraints to greater use'. *Plant Physiology* 131; 872–877.
- [13] Hai, S., Oura, H. and Nakajima, T. (1996). "Colour reaction of some sapogenins and saponin with vanillin and sulphuric acid". *Planta Med. Za.*, 116 122.
- [14] Hsu, H. W., Vavak, L. D., Satterlee, E. and Miller, G. A. (1977). "A multi enzyme technique for estimating protein digestibility". *Journal of Food Science* 42: 1269–1273.
- [15] Hurrel, R. F., Reddy, M.B., Juillerat, M. and Cook, J.D. (2003). "Degradation of phytic acid in cereal porridges improves iron absorption by human subjects". *American Journal of Clinical Nutrition* 77(5); 1213–1219.
- [16] Kakade, M. C., Simons, N. and Licenser, L. E. (2009). "An evaluation of natural vs synthetic substrates for measuring the antitryptic activity of soybean samples". *Cereal Chemistry* 46; 518–526.



- [17] Machaiah, J. P., Pednekar, M. D. and Thomas, P. (1999). "Reduction in flatulence factors in mung bean (*Vigna radiata*) using low dose irradiation. *Journal of the Science of Food and Agriculture* 79: 648-652.
- [18] Markker, H. P. S. (1994). Quantification of tannins: A laboratory manual. *International Centre for Agricultural Research in Dry Area (ARDA)*, Aleppo, Syria.
- [19] Markker, H. P. S. and Berker, K. (1997). "Nutrients and antiquality factors in different morphological parts of the *Moringa oleifera* tree". *J. Agric. Sci.* 128; 311–322.
- [20] Nwosu, J. N. (2006). "The effects of blanching and cooking on the antinutritional properties of *Bosqueia angolensis* seed". Proceeding of the 30th annual conference of the Nigerian Institute of Food Science and Technology, Lagos, 68-69.
- [21] Ojo, M. A, Ade-Omowaye, B.I.O. and Ngoddy, P. O. (2014). "Nutrients and phytochemical profiles of some selected underutilized hard-to-cook legumes in Nigeria". *The International Journal of Science & Technoledge* 2 (8); 108-114.
- [22] Ojo, M. A, Ade-Omowaye, B.I.O. and Ngoddy, P. O. (2016). "Mineral elements in Canavalia ensiformis: influence of hydrothermal processing techniques". Annals. Food Science and Technology 17 (2): 548-555.
- [23] Okorie, S. U., Ebiringa, D. C. and Durumba, C. N. (2004). "Effects of soaking and malting on the chemical composition and functional properties of soybean (*Glycine max*)". in Elemo, G. N. (Ed). Nigerian Institute of Food Science and Technology 2004 proceedings of the 30th conference, Lagos, 196-197.
- [24] Oyebode, E. T., Ojo, M. A. and Oshodi, A. A. (2007). "Physico chemical properties and *in vitro* protein digestibility of flours and protein isolate from *Adenopus breviflorus* Benth seed". *Science Focus* 12(1); 28-34.
- [25] Paedes-Lopez, O. and Harry, G. I. (2009). "Changes in selected chemical and antinutritional components during tempeh preparation using fresh and hardened common beans". *Journal of Food Science* 54; 968-970.
- [26] Polhill, R. M. (1994). Classification of the *Leguminosae*. In Phytochemical Dictionary of the Leguminosae (Bisby. F.A.; Bucking-harm, J. and Harbone, J.B., eds.). Chapman and Hall, New York, 152 157.
- [27] Reyden, P. and Selvendran, R. R. (1993). Phytic properties and determination. In R. Macrae, R. K.

- Robinson and M. I. Sadler (Eds.). Encyclopedia of Food Science, Food Technology and Nutrition, Academic Press, London, 3582-3587.
- [28] SAS (2005). Stastistic Package for Social Science (version 15.0). SAS Institute Inc., Cary, NC.
- [29] Seaman, J. C., Hutchison, J. M., Jackson, B. P. and Vulava, V. M. (2003). "Antinutrients in foods a review". *Journal of Environmental Quality* 32(1); 153 161.
- [30] [30] Shamsuddin, A. M. (2002). "Anticancer functions of phytic acid". *International Journal of Food Science and Technology* 37; 769-782.
- [31] Siddhuraju, P. and Becker, K. (2001). "Effect of various domestic processing methods on antinutrients and *in vitro* protein and starch digestibility of two indigenous varieties of Indian tribal pulse, *Mucuna pruriens var. utilis*". *Journal of Agricultural Food Chemistry* 49; 3058–3067.
- [32] Smith, C., Megen, C., Megen, W. V., Twaalfhaven, L. and Hitchock, C. (2000). "The determination of trypsin inhibitor levels in foodstuffs". *Journal of* the Science of Food and Agriculture 34; 341–350.
- [33] Udensi, E. A., Ansa, N. U. and Maduka, M. (2008). "Effect of processing methods on the level of some antinutritional factors in *Mucuna flagellipes*". *Nigerian Food Journal* 26; 53-59.
- [34] Vijayakumani, K., Pugalenthi, M. and Vadivel, V. (2007). "Effects of processing methods on the levels of antinutrients and *in vitro* protein digestibility of *Bauhinia purpurea L.* seeds. *Food Chemistry* 59; 367–371.
- [35] Vindiolla, O. L., Seib, P. A. and Hoseney, R. C. (1996). "Accelerated development of the hard-to-cook state in beans". *Cereal Foods World* 31; 538–552.
- [36] Wang, N., Hatcher, D. W., Toews, R., Gawalko, E. J. (2009). "Influence of cooking and dehulling on nutritional composition of several varieties of lentil (*Lens culnaris*)". *LWT*, 42; 842-848.
- [37] Xu, B. and Chang, S. K. C. (2008). "Effect of soaking, boiling, and steaming on total phenolic content and antioxidant activities of cool season food legumes". *Food Chemistry* 110 (1-13).
- [38] Xu, B. and Chang, S. K. C. (2009). "Phytochemical profiles and health-promoting effects of coolseason food legumes as influenced by thermal processing'. *Journal of Agricultural Food Chemistry* 57: 10718-10731.