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Processing effects of soaking and hydrothermal methods on the components and *in vitro* protein digestibility of *Canavalia ensiformis*

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Abstract

After aqueous soaking to varying hydration levels, seed of *Canavalia ensiformis* was subjected to four hydrothermal processing techniques (atmospheric boiling, atmospheric steaming, pressure boiling and pressure steaming). Influences of hydrothermal techniques on the nutrients, antinutritional components and protein digestibility were studied. Aqueous soaking before hydrothermal treatments induced reduction in cooking time. Nutrients in the legume are better conserved when boiled at elevated pressure. The processing techniques caused significant reduction ($p<0.05$) in the various antinutritional components. Highest *in vitro* protein digestibility of 85.73% was observed after the legume seed was boiled at elevated pressure.

Introduction

The continuous increase in population and inadequate supply of protein have inadvertently increased the occurrence of protein energy malnutrition (PEM) in many developing nations of the world. PEM is a major nutritional syndrome that is affecting more than 200 million children and nursing mothers in many areas of the world (Trustwell, 2003). Animal proteins are usually superior in quality to plant protein and the former are relatively more expensive and out of reach to the large percentage of the people in developing countries such as Nigeria. Among plants, legumes are the most widely used sources of plant protein. Legumes are often advocated in diets because of their nutritional benefits and they are a cheap source of protein (Muzquiz *et al*., 1999). In recent years, they are recommended as health promoting foods by health organisations and dieticians. Legumes contain varying concentrations of carbohydrates, lipids, proteins, and vitamins. Legumes contain antinutritional components that must be inactivated if their full value is to be realised. These include saponin, heamaglutinin, cyanogenic glycoside, phytic acid, trypsin inhibitors, tannin etc. These antinutritional components interfere with normal growth by preventing efficient utilisation of nutrients (Reddy and Sathe, 2002).

Canavalia ensiformis is one of the underutilised legumes in South West Nigeria. It is planted by peasant farmers for subsistence purposes. In addition to the presence of antinutritional components, another limiting factor to its utilisation is the hard-to-cook nature. Locally, the use of tenderiser such as trona is employed to solve the problem of prolong cooking but the safety limit of such treatment as well as its effect on human physiology are yet to be ascertained. There is paucity of information on the components of *Canavalia ensiformis*. In this study, combination of soaking with other hydrothermal processing methods have been employed to reduce cooking time. Also, information on the effects of processing on the nutrients and antinutrients is limited. Therefore, efforts have been made in this study to investigate the effects of soaking and hydrothermal processing methods on the nutirients, antinutritional components and protein digestibility of *Canavalia ensiformis.*

Materials and Methods

Sample and preparation

The seeds of *Canavalia ensiformis* were purchased from Sanngo Market, Saki, Oyo State, Nigeria. The seeds were dry-cleaned. Particles such as stalks, pebbles, immature and broken seeds were removed. They were then packaged in labelled plastic containers. Some seeds were grinded in a Kenwood Blender to obtain flour by placing them in

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the grinding jar with stainless steel blades fitted to the rotor of the blender. The flour was then packed in an air tight cellophane bag and stored at ambient temperature for subsequent analysis.

Soaking and determination of soaking time

Soaking and determination of hydration rate of the sample were carried out using the method described by Xu and Chang (2008) with slight modification. The sample (500 g) was cleaned and soaked in 2500 cm^3 of distilled water in a glass jar at ambient temperature 25±3°C for up to 24 h. Water absorption (increase in moisture) of the legume during soaking was measured hourly for the initial 0-6 h and every two hours. The soaked legumes was blotted with a woollen hand towel at appointed time to remove excess water before weighing and returning back into soaking water. Moisture content of the soaked legume was calculated based on weight differences after water absorption. Furthermore, the water absorption curve was plotted to show the kinetic increase of the moisture content with time. The plateau phase of water absorption curve was defined as 100% hydration level. Soaking time of the legume with desired hydration level was calculated through polynomial equation of respective water absorption curve.

For the subsequent boiling and steaming experiments, the legume was pre-soaked to the desired hydration levels of 0, 10, 25, 50, 75 and 100% by controlling soaking time. The soaked seeds were then drained and boiled or steamed by the following methods:

Boiling at atmospheric pressure (BAP)

Atmospheric boiling under normal atmospheric pressure of the sample was done using the procedure described by Xu and Chang (2008). The boiling of each of the legume samples was conducted using a domestic cooker (Tower, TG). Pre-soaked samples (500 g) with varying hydration levels were boiled in water. Determination of cooking time for the atmospheric boiling of these samples was conducted by tactile method (Vindiolla *et al.*, 1996) in which the cooked sample was squeezed between the forefinger and thumb with moderate pressure. A seed was considered to be cooked when it could be squeezed by finger easily. Cooking time was defined as the time duration, in minutes, of at least 90% of seeds subjected to cooking. After boiling treatments the seeds were drained and both the cooking water and the drained seeds were cooled in plastic containers. Subsequently the cooked seeds and cooking water were dried at 45±5°C using cabinet drier. The dried samples were stored in plastic containers prior to analysis.

Boiling at elevated pressure (BEP)

Pressure boiling was performed using a domestic pressure cooker (Binatone PC-5001) at about 80±8 KPa. Five fold of distilled water was added to the pre-soaked legume (500 g) at varying hydration levels as described under atmospheric boiling in a glass flask which was covered with aluminium foil. The content of the flask was brought quickly to boiling on a hot plate. The legume samples with boiling water was placed into pre-heated pressure cooker with 2500 cm^3 of boiling water and the lid was locked in place. The cooking time was counted from when steam began to spurt out from pressure lid. Cooking time was determined by tactile method (Vindiolla *et al*., 1996). When the samples have been boiled under pressure to the desired cooking time, the pressure cooker was then removed from the heat source and the pressure released. Boiling water and the boiled legume samples were cooled to room temperature 25±3°C and dried at 45±5°C using a cabinet drier. The dried samples were then stored in a plastic container before analysis.

Steaming at atmospheric pressure (SAP)

Steaming and determination of steaming time were carried out at normal atmospheric pressure using steam cooker. The pre-soaked legume samples (500 g by weight) with varying hydration levels were placed on a tray in the steam cooker covered with lid and were steamed over 2500 cm^3 of boiling water. Steaming times were determined by tactile method (Vindiolla *et al*., 1996). After the steaming process, legumes were cooled and dried at 45±5°C in a cabinet drier. The dried samples were then stored in a plastic container before analysis.

Steaming at elevated pressure (SEP)

Steaming under pressure was performed using a pressure cooker (Binatone PC-5001) at about 80±8 KPa. Pre-soaked samples (500 g by weight) of varying hydration levels were placed on a tray in a pressure cooker and steamed over boiling water under selected high pressure (80±8 KPa). Steamed samples were placed in plastic containers, cooled and then dried at 45±5°C in a cabinet drier. The dried samples were stored in plastic containers before analysis.

Determination of nutrient composition

The proximate composition of unprocessed and processed samples were determined using standard methods (A.O.A.C., 2005). Analyses were carried

out for crude protein, crude fat (ether extract), crude fibre, total ash and moisture content. The total carbohydrate was estimated by difference.

Determination of anti-nutritional factors

Determination of trypsin inhibitor activity

The trypsin inhibitor activity (TIA) 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.

About 1 g of finely ground and sieved sample of the seeds was defatted for 3 h using n-hexane. The sample was mixed with 50 cm^3 of 0.01M sodium hydroxide (NaOH) and the pH was adjusted to 9.5 using 0.1M NaOH or 0.1M hydrochloric acid (HCl). The mixture was macerated in warming blends for 2 min and centrifuged for 10 min at 100 rpm. The extract from each sample was diluted with distilled water to obtain a dilution whereby 1 cm^3 extract produced trypsin inhibition activity of between 40 and 60% and such dilution was used.

Each sample dilution was used with BAPNA substrate and trypsin solutions as described by Kakade *et al*. (2009) at 37ºC. The reaction was allowed to take place in a water bath (Uniscope model SM 902B) for 10 min and the absorbance read at 410 nm against each sample blank.

Trypsin inhibitor was calculated as

$$TIA = [2.632 \times D \times A_1] / S = mg\ pure trypsin/g\ sample$$

where

D= dilution factor

A1= change in absorbance (pure trypsin and sample extract)

S= sample mass (g)

Determination of tannin content

The tannin content of the legume seed was determined by modifying the procedure of Makkar (1994). The seed flours were defatted using diethyl ether, ground and sieved through 500 µm sieve. About 0.2 mg of the defatted flour was extracted with 10 cm^3 of 70% aqueous acetone for 2 h in a water bath (Uniscope model SM 902B) at 30ºC. The extract was centrifuged at 3500 rpm for 20 min and 0.05 cm^3 of the supernatant was used. Increasing concentration of standard tannic acid was prepared and 0.5 cm^3 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. The regression equation was

$$Y = 0.021X - 0.01$$

where

Y = absorbance

X = tannic acid (µg)

Tannic acid from each sample was determined and expressed as mg/g of the flour sample.

Determination of total saponin content

The procedure of Makkar and Becker (1997) was modified for use. About 0.5 g of the dried, grinded legume sample was defatted with 10 cm^3 of petroleum ether by shaking for 4 h and then the residue was extracted twice with 5 cm^3 of aqueous methanol on an orbit shaker by shaking for 4 h each. The extract was stored at 40^0C in the dark for use.

The total saponin content (TSC) was determined using the spectrophotometric method (Fagbemi *et al*., 2005). About 0.1 cm^3 of the legume extract, 0.4 cm^3 of 80% methanol solution, 0.5 cm^3 of freshly prepared vanillin solution (in ethanol) and 50 cm^3 of 72% sulphuric acid were mixed together thoroughly in an ice water bath. The mixture was warmed in a water bath at 60^0C for ten minutes and then cooled in ice cold water. Absorbance at 544 nm was recorded against the reagents blank with a UV-visible spectrophotometer (UV 160 Shimadzu). The results were expressed as mg of soya saponin equivalent/g of legume (mg/g) 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.

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 cm^3 of petroleum ether by shaking for 4 h and then the residue was extracted with 10 cm^3 of 24% HCl by shaking on the orbit shaker for 6 h. The extract was stored at 4ºC in the dark prior to further analysis.

Phytic acid was determined using the colourimetric (Wade reagent) method described by Gao *et al.* (2007) with slight modification. About 0.1 cm^3 of the extract was diluted by 29 cm^3 of distilled water, and then 3 cm^3 of this diluted sample

was combined with 1 cm^3 of freshly prepared Wade reagent (0.03%, $FeCl_3 . 6H_2O$ + Sulfosalicylic acid) in a 15 cm^3 tube. The contents were thoroughly mixed and centrifuged at 5500 rpm at 10ºC for 10 min. A series of calibration standards containing 0, 5, 10, 20, 25, 75 or 100 mg/cm^3 of phytic acid were prepared by diluting 10 mg/cm^3 of phytic acid stock solution with distilled water. Absorption of colour reaction products for both samples was read at 500 nm on a UV Spectrophotometer (UV160 Shimadzu) against water as blank. The results were expressed as milligrams of phytic acid per gram of legume (mg/g) on a dry weight basis.

In vitro *multi-enzymes protein digestibility (IVPD) determination*

The IVPD of the seed flours was determined using the procedure of Hsu *et al.* (1977). The enzymes used were porcine pancreatic trypsin (Z.F 93615.0025), borine pancreatic chymotrypsin (Z.F 27270) and porcine intestinal peptidase (Z.F 77163.0500) manufactured by Zefa Laboratory Service, GMBH Germany. The activity of the enzymes was initially determined before use by using them to digest casein. Each of the samples was ground to fine powder and sieved. Appropriate grammes of each of the flour samples were each dissolved in 50 cm^3 distilled water to give sample suspension of 6.25 mg protein/cm^3. Each sample suspension was adjusted to pH 8 and incubated in water bath at 37ºC with constant stirring.

Fresh multi-enzyme solution was prepared to contain 1.6 mg trypsin, 3.1 mg chymotrypsin and 1.4 mg peptide dissolved in 1 cm^3 distilled water. The pH of enzyme solution was maintained at 8. 5 cm^3 of multi-enzyme solution was added to each sample suspension with constant stirring at 37ºC. The pH of each sample suspended was recorded at 10 min and 15 min after adding the enzyme solution. The IVPD was calculated using the equation proposed by Hsu *et al.* (1977).

$$Y = 210.464 - 18.10X$$

where

Y = *in vitro* protein digestibility (%)

X = pH of sample suspension after 10 min and 15 min

Statistical analysis

Analyses were carried out in three replicates and expressed as mean data ± SD (standard deviation). All data were subjected to Analysis of Variance [ANOVA] and the significant differences were determined at $p<0.05$. The means were separated by Duncan's multiple-range tests.

Results and Discussion

Soaking and hydrothermal processing effects on cooking times of the legume samples

The influence of soaking followed by processing using different hydrothermal techniques at varying hydration levels are presented in Table 1. The corresponding volumes of water used for each cooking operation are also presented. At 0% hydration level i.e, without soaking, the cooking time ranged from 60 minutes for BEP to 185 min for SAP. Similar results were observed when the sample at 10% hydration level was cooked using the different hydrothermal processing methods. The similarity between the cooking times of the hydrothermal processing methods at 0% (raw sample without soaking) and 10% hydration level was due to the fact that hydration of the seeds to 10% level was in all probability insufficient to cause the seeds to absorb enough water needed to effect softening prior to cooking. There was further reduction in the cooking time at 25, 50, 75, and 100% hydration levels. At 50% hydration level, for instance, percentage reductions in cooking time were 17.86, 7.89, 60.00 and 48.57% for BAP, SAP, BEP and SEP, respectively. The highest percentage reduction of 64.29 was observed at 100% hydration level when the legume was boiled at elevated temperature.

Generally, the volume of water required for cooking decreased with increase in the hydration level. This was true for each of the processing methods. Cooking of the legume at 0% hydration level required much more water than at 100% hydration level (Table I). There was decrease in the cooking time as the level of hydration increased. Processing at elevated pressure was observed to show a better reduction in the cooking time than processing at normal atmospheric pressure. Moreover, boiling at elevated pressure (BEP) was observed to induce better reduction in the cooking time than steaming at elevated pressure (SEP). Increase in pressure causes cooking temperature to rise, thereby making foods to cook faster (Elkins, 1999; Kivosto *et al*., 2006).

Proximate components of the pre-soaked and processed legume samples

Processing of the legume using different hydrothermal techniques had significant effect ($p<0.05$) on the proximate composition. The protein content of the dried raw sample of *C. ensiformis* was 19.95%. As reflected in Table 2, boiling at BAP caused a change of 10.32% reduction in protein content. Soaking of the legume at varying hydration levels resulted in decrease in percentage protein.

Table 1. Effect of hydrothermal processing on cooking times of *Canavalia ensiformis* at varying hydration levels.

Hydration levels (%)	Volume of H_2o used (cm^3)				Cooking times (min)			
	BAP	SAP	BEP	SEP	BAP	SAP	BEP	SEP
0	2000 ±28.28^c	2550±42.46^e { 21.57}*	1200±0.00^a { 40.00}	1440±14.14^d {28.00}	140±0.00^c	185± 7.00^e {24.32}*	60± 0.00^a {57.43}	78±2.82^e {44.29}
10	2000±0.00^e { 0.00}	2550±50^e {21.57}*	1200±20^b {40.00}	1440±40^d {28.00}	140±2.82^c { 0.00 }	184±0.00^e {23.91}	60±0.00^a {57.14}	78±1.44^e {44.29}
25	2000±42.42^e {0.00}	2550±70.00^e {21.57}*	1000±0.00^a {50.00}	1190±70.07^b {40.50}	130±1.41^c {7.14}	170±0.71^d {17.65}*	58±0.71^a {58.57}	75±0.00^b {46.42}
50	1930±21.21^c {3.50}	2450±7.07^d {18.37}*	950±21.21^a {52.50}	1140±28.28^b {43.00}	115±1.41^c {17.86}	152±1.41^d { 7.89}*	56±0.00^a {60.00}	72±1.41^b {48.57}
75	1800±28.28^c {10.00}	2300±67.18^d {13.04}*	800±0.00^a {60.00}	950±14.14^b {52.50}	110±0.00^c {21.43}	144±1.42^d {2.78}*	52±2.12^a {62.86}	66±0.00^b { 52.86}
100	1700±0.00^c {15.00}	2220±14.14^d {9 .91}*	800±0.00^a { 60.00}	920±21.21^b { 54.00}	91±0.71^c {35.00}	120±0.00^d {14.29}	50±0.00^a {64.29}	58±0.71^b {58.57}

Values are means ± standard deviation (n=3) on dry basis; means with different letters on the same row are significant (p<0.05).
Values in parenthesis represent percentage reduction. Values with * represent percentage increase.
BAP=boiling at normal atmospheric pressure; SAP=steaming at normal atmospheric pressure; BEP=boiling at elevated pressure; SEP=steaming at elevated pressure

For instance, at 50, 75 and 100% hydration levels, atmospheric boiling caused protein reduction by 6.77, 4.57 and 4.19%, respectively. In general, with increasing hydration level the percentage reduction in protein content decreased. This was true for all the processing methods. Hydrothermal processing of the legumes at elevated pressure caused better retention in the concentration of protein.

The relatively low percentage reduction in protein content during processing at elevated pressure might not be unconnected with the fact that increase in pressure during cooking causes decrease in cooking times. Decrease in cooking time minimises protein leaching into the cooking water (Adewusi and Osuntogun, 1991). The decrease in protein content during hydrothermal processing is in agreement with the work on Albazzia lebbech (Abdullahi *et al*., 2007). As shown in Table 2, BEP caused better retention of protein than steaming. This is because boiling involved soaking during which the legume seeds absorb water and thus increase the hydration level. Increasing the hydration level, as earlier observed, decreased the cooking time and hence minimized leaching.

All the hydrothermal processing methods had significant effects (p<0.05) on the total ash. The total ash content of the dry raw sample of 3.23% was reduced by 32.50, 34.36, 17.02 and 19.50% after processing by BAP, SAP, BEP, and SEP, respectively at 0% hydration level. At varying hydration levels, the legume seed exhibited varying degrees of percentage reduction in ash content. At 75% hydration level, BEP and SEP caused 3.41 and 6.81% reduction, respectively. Reduction of total ash during processing was due to leaching. Similar reduction was observed for *Vigna unguiculata* after boiling (Ojo and Ajayi, 2005). Fagbemi (2007) also reported reduction in the total ash content of *Telifaria occidentalis* during boiling. Loss of ash was minimised during processing by drying the cooking water along with the legume seeds. The oil content of *C. ensiformis* was low (1.91%). Hydrothermal processing did not induce remarkable change in the oil content. The oil content of the legume before and after hydrothermal processing was low and hence does not qualify as oil seed. The moisture content of the raw and processed samples was low. Moisture content of 14.03 and 14.05% were reported for Bambara groundnut and lima bean, respectively. (Fasoyiro *et al.*, 2006). Low level of moisture content provides a good indication of stability on storage.

The hydrothermal processing methods caused significant reduction (p<0.05) in the crude fibre content of the legume at varying hydration levels. Without soaking (i.e. 0% hydration level) the raw sample with the fibre content of 3.56% decreased by 17.13% and 15.73% after processing by boiling and steaming at atmospheric pressure, respectively while boiling and steaming at elevated pressure reduced the fibre level by 9.83 and 10.11%, respectively. Fagbemi (2007) reported a similar observation in which the crude fibre content of *T. occidentalis* 2.60%, decreased to 2.49% after boiling. The change in the crude fibre content of the legume was probably due to

Table 2. Effect of hydrothermal processing methods on proximate composition of *Canavalia ensiformis* at varying hydration levels.

Hydration levels	Proximate component (%)	RS	BAP	SAP	BEP	SEP
0%	Moisture content	11.30±0.65^a	9.46±0.42^d {16.28}	9.18±0.40^c {18.76}	8.61±0.31^b {23.80}	8.16±0.20^a {25.13}
	Ash	3.23±0.28^a	2.18± 0.00^b {32.50 }	2.12±0.00^a {34.36}	2.68± 0.21^a {17.02}	2.60± 0.02^c {19.50}
	Crude protein	19.95±0.64^a	17.89± 0.60 ^{b} {10.32}	17.57± 0.40^a {11.92}	18.65± 0.60 ^{d} {6.51}	18.52± 0.41^c {7.16}
	Ether extract	1.91± 0.03^d	1.86± 0.01^b { 2.61}	1.70± 0.01^a {10.99}	1.87± 0.60^c {2.09}	1.87± 0.01^c {2.09}
	Crude fibre	3.56± O.15^a	2.95± 0.20^a {17.13}	3.00 ± 0.02^a {15.73}	3.21± 0.10^c {9.83}	3.20± 0.10^c {10.11}
	Carbohydrate	60.05± 1.63^a	65.66± 0.91^d {8.54}	67.70± 0.81^a {11.64}	64.96± 1.34^b {7.56}	65.65±1.21^c {8.53}
	Total dry matter	88.70± 0.96^a	90.54± 0.91^b {2.03}	90.82± 1.01^c {0.74}	91.39± 1.00^d {0.95}	99.94± 1.00^a {0.81}
10%	Moisture content	11.30±0.65^a	9.49±0.08^d { 16.02 }	9.20± 0.04^c { 18.58 }	8.58± 0.40^b { 24.07 }	8.15± 0.12^a { 27.88 }
	Ash	3.23±0.28^a	2.18± 0.01^b { 32.51 }	2.12± 0.10^a { 34.37 }	2.79 ±0.20^c {13.62 }	2.75± 0.03^c {14.86 }
	Crude protein	19.95±0.64^a	17.89±0.59^b { 10.33 }	17.57± 0.41^a {11.93 }	18.65±0.58^d { 6.52 }	18.52±0.41^c { 7.17 }
	Ether extract	1.91± 0.03^a	1.86± 0.01^b { 2.62 }	1.70± 0.01^a {10.99 }	1.89± 0.02^d { 1.05 }	1.87± 0.41^c {2.09 }
	Crude fibre	3.56±O.15^a	2.95± 0.03^a { 17.13 }	3.00± 0.02^b { 15.73 }	3.38 ± 0.11^d { 5.06}	3.20± 0.10^c { 10.11 }
	Carbohydrate	60.05± 1.63^a	65.65±0.84^d { 8.50 }	66.44± 0.46^a {9.62 }	64.71± 1.06^b { 7.20 }	65.51± 1.05^c { 8.33 }
	Total dry matter	88.70± 0.96^a	90.51± 1.08^b { 2.00 }	90.80±1.01^c { 2.31 }	91.42±1.01^d {2.98 }	91.85± 1.50^a { 3.43 }
25%	Moisture content	11.30±0.65^a	9.51± 0.14^d {15.84 }	9.19± 0.07^c {18.67}	8.57± 0.32^b { 24.16}	8.17± 0.12^a {27.70}
	Ash	3.23± 0.28^a	2.40± 0.01 ^{c} {25.70}	2.39± 0.01 ^{a} {26.01}	2.90± 0.16^c { 10.22}	2.75± 0.02^c {14.86}
	Crude protein	19.95± 0.64^a	17.89± 0.71^b {10.33 }	17.59± 0.39^a { 11.83}	18.67± 0.56^d { 6.42}	18.55± 0.36^c {7.02}
	Ether extract	1.91± 0.03^d	1.87± 0.00^b {2.01}	1.69± 0.01^a { 1.32}	1.90± 0.01^d { 0.52}	1.89± 0.02^c { 1.05}
	Crude fibre	3.56± O.15^a	3.22± 0.16^b {9.55 }	3.00± 0.02^a { 15.73 }	3.40± 0.15^d { 4.49}	3.36± 0.15^c { 5.62}
	Carbohydrate	60.05±1.63^a	65.11±1.31^c {17.77}	66.14± 0.71^a {9.21}	64.56±1.23^b { 6.99}	65.28±1.12^d { 8.01}
	Total dry matter	88.70± 0.96^a	90.49± 1.21^b {1.98 }	90.81± 0.99^c {2.32}	91.43± 1.00^d { 2.99 }	91.83± 1.01^a {3.41}
50%	Moisture content	11.30±0.65^a	9.32± 0.01^d {17.61}	9.21± 0.03^c { 18.50}	8.58± 0.43^b { 24.25}	8.20±0.05^a {27.43}
	Ash	3.23±0.28^d	2.43± 0.03^b {24.77 }	2.39± 0.01^a {26.01}	2.93± 0.20^a {9.28}	3.00± 0.01^c {7.12 }
	Crude protein	19.95± 0.64^a	18.60± 0.38^c { 6.77}	18.29± 0.50^a { 8.32 }	19.20± 0.64^d { 3.76 }	18.55± 0.42^b {7.02 }
	Ether extract	1.91± 0.03^d	1.87± 0.01^b { 2.09}	1.70± 0.00^a { 11.00}	1.91± 0.02^d { 0.00 }	1.89± 0.01^c { 1.05 }
	Crude fibre	3.56± O.15^a	3.27± 0.14^b {8.15 }	3.02± 0.01^a { 15.17}	3.46± 0.13^d {12.8 }	3.45± 0.15^c { 3.09}
	Carbohydrate	60.05± 1.63^a	64.51± 1.22^c { 6.91}	65.39 ± 0.95^a { 8.17 }	64.02±1.12^b { 6.20 }	64.91±1.04^c { 7.49 }
	Total dry matter	88.70± 0.96^a	90.68± 0.95^b { 2.18}	90.72± 1.00^c {2.23 }	91.42± 1.02^d { 2.96 }	91.88±1.10 ^{a} { 3.46 }
75%	Moisture content	11.30±0.65^a	9.35± 0.04^d { 17.26 }	9.21± 0.03^c { 18.50 }	8.54± 0.30^b { 24.42 }	8.15± 0.10^a { 27.88 }
	Ash	3.23±0.28^a	2.84±0.03^b {12.07 }	2.65± 0.02^a { 17.96 }	3.12± 0.18^c { 3.41 }	3.01± 0.02^c { 6.81 }
	Crude protein	19.95± 0.64^a	19.05± 0.21^b { 4.51 }	18.75± 0.42^a {6.02 }	19.55± 0.43^d { 2.01 }	19.40± 0.38^a { 2.76 }
	Ether extract	1.91± 0.03^c	1.90± 0.02^b { 0.53 }	1.71± 0.01^a { 10.47 }	1.91± 0.01^c { 10.00 }	1.90± 0.02^b { 0.53 }
	Crude fibre	3.56± O.15^a	3.22± 0.11^a {9.55 }	3.30± 0.02^b { 7.30 }	3.47± 0.12^c { 2.53 }	3.47± 0.16^c { 3.53 }
	Carbohydrate	60.05±1.63^a	63.64±1.00^c { 5.64 }	67.68± 1.20^a { 11.27 }	63.41±1.12^b {5.30 }	64.07± 1.01^d { 16.27 }
	Total dry matter	88.70±0.96^a	90.65± 0.71^b { 2.15 }	90.79±1.01^c { 2.30 }	91.46± 1.00^d {3.02 }	91.85± 1.05^a { 3.43 }
100%	Moisture content	11.30± 0.65^a	9.35± 0.04^d { 17.26 }	9.21± 0.03^c {18.50 }	8.54± 0.30^b { 24.42 }	8.15± 0.10^a {27.88 }
	Ash	3.23±0.28^a	2.84±0.03^b {12.07 }	2.65± 0.02^a {17.96 }	3.12± 0.18^c {3.41 }	3.01± 0.02^c {6.81 }
	Crude protein	19.95± 0.64^a	19.05± 0.21^b {14.51 }	18.75± 0.42^a {6.02 }	19.55± 0.43^d {2.01}	19.40± 0.38^c {2.76 }
	Ether extract	1.91± 0.03^c	1.90± 0.02^b { 0.51 }	1.71 ± 0.01^a {10.47 }	1.91± 0.01^c { 0.00 }	1.90± 0.02^b { 0.52 }
	Crude fibre	3.56± O.15^d	3.22± 0.11^a {9.55 }	3.30± 0.02^b {7.30 }	3.47± 0.12^c { 2.53 }	3.47± 0.16^c { 2.53 }
	Carbohydrate	60.05± 1.63^a	63.64± 1.00^c {5.64 }	67.68±1.20^a {11.27 }	63.41± 1.12^b {5.29 }	64.07± 1.01^d {6.27 }
	Total dry matter	88.70± 0.96^a	90.65± 0.71^b {2.15 }	90.79±1.01^c {2.30 }	91.46± 1.00^d {3.01 }	91.85± 1.05^a {3.42 }

Values are means ± standard deviation (n=3) on dry basis; means with different letters on the same row are significant (p<0.05).
Values in parenthesis represent percentage change in concentration after processing.
RS =raw dried sample; BAP=boiling at normal atmospheric pressure; SAP=steaming at normal atmospheric pressure; BEP=boiling at elevated pressure; SEP=steaming at elevated pressure.

modification of texture of plant tissue caused by the release of carbonyl groups in the pectin molecules during hydrothermal processing (Montanez-Saenz *et al*., 2003; Ojo, 2011).

Antinutrtional components of the pre-soaked legume samples

The changes in the concentration of anti-nutritional components before and after soaking at varying hydration levels are presented in Table 3. Soaking generally reduced each of the anti-nutrients in the legume. The phytic acid content of the raw sample was 59.28 mg/g. After soaking, the percentage reduction in the phytic acid content ranged from 1.21 to 6.87%. The percentage reduction obtained were comparable to but lower than the value reported for *Mucana flagellipes.* Udensi *et al.* (2008) reported 27.9% of *M. flagellipes* after soaking for 6 h and 36.0% reduction after soaking for 24 h at room temperature. In another study, *S. rostrata*

Table 3. Concentration of antinutrtional components (mg/g) in *C.ensiformis* before and after soaking at varying hydration levels (mg/g)

Antinutrtional components	Hydration level (%)					
	0	10	25	50	75	100
Phytic acid	59.28± 0.26^{f}	58.56± 0.10^{e} {1.21}	58.09± 0.28^{e} {2.01}	56.67± 0.55^{c} {4.40}	55.34±0.41^{b} {6.65}	55.21± 0.07^{a} {6.87}
Saponin	5.29± 0.33^{c}	5.26± 0.10^{c} {0.57}	5.22±0. 20^{c} {1.32}	5.09± 0.14^{a} {3.78}	5.09±0. 13^{a} {3.78}	5.09±0.21^{a} {3.78}
Trypsin inhibitor	25.61± 0.21^{f}	25.07± 0.06^{e} {2.11}	24.13± 0.31^{d} {5.78}	23.49± 0.41^{c} {8.28}	21.87± 0.43^{b} {14.60	21.21± 0.05^{a} {17.18}
Tannin	27.47± 0.44^{f}	26.50± 0.12^{e} {3.53}	26.37± 0.21^{e} {4.00}	26.12± 0.03^{c} {4.91}	24.70±0.10^{b} {10.08}	23.68± 0.11^{a} {13.79}

The values are mean ± standard deviation (n = 3) on dry basis; means with different letters in the same row are significantly different (p<0.05). Values in parenthesis represent the percentage loss.

also experienced 9.7% reduction in the phytic acid after soaking (Siddhuraju *et al.,* 2002). Decrease in the phytic acid content was mainly due to leaching (Siddhuraju and Becker, 2001). Loss of phytic acid during soaking can also be attributed to degradation of the phytate molecule (Vijayakumani *et al.*, 1997).

The percentage reduction in saponin content increased with increasing hydration levels. At hydration levels of 50%, 75% and 100%, *C. ensiformis* exhibited a pattern of decrease in saponin content by 3.78%. This reduction agrees with previous reports on species of an unconventional legume – Sesbania (Siddhuraju *et al.*, 2002). At varying hydration levels, percentage reduction of trypsin inhibitor ranges from 2.11% to 17.18%. Similar observations were reported when *Prosopis africana,* was processed (Odo *et al*., 2004).

The percentage reduction in tannin content ranged from 3.53% to 13.79%. This reduction is similar but comparatively lower than that of the earlier study of *Mucuna flagellipes* which ranged from 58.4% at 6 h to 74.9% at 24 h of soaking (Udensi *et al.*, 2008). Reduction in tannin might be due to leaching out of the polyphenonls into the soaking water.

Antinutrtional components of the processed legume samples

Table 4 shows the effects of hydrothermal processing methods on the antinutritional components of *C. ensiformis*. The processing methods had significant effects on the anti-nutritional components studied; the percentage reduction depended on the processing methods and the duration of the heating. In the raw sample, phytic acid was present at concentration of 59.28 mg/g. Boiling and steaming at normal atmospheric led to the reduction by 63.26% and 57.02%, respectively while boiling and steaming at elevated pressure led to the percentage reduction by 62.97 and 56.41%, respectively. In an earlier results obtained by Elhardalon and Walker (1994), similar percentage reduction of 60.5% in phytic acid was reported when lentil was cooked. Reduction range of 22.40-43.37% was observed when *M. flagellipes* was boiled at varying time intervals. These variations in results may be due to differences in the types, their sources and inadvertent variations that might have existed in the processing procedures. Loss of phytic acid during hydrothermal processing may be due to chemical degradation of phytic acid to inositol hexaphosphate hydrolysed to penta and tetraphosphates. It may also be due to formation of insoluble complexes between phytic acid and food components such as protein and mineral elements (Siddhuraju *et al*., 2002). Moreover, partial dissociation of phosphorus from the structure of phytic acid ($C_6H_{18}O_{24}P_6$) may also be responsible for the decrease that took place during hydrothermal processing. Although phytic acid is often considered as an anti-nutrient in legumes because of its ability to form complexes with protein and some minerals, recent studies have shown that low levels of phytic acid had health benefits as antioxidants (Xu and Chang, 2009). Phytic acid has also been reported to assist in controlling hypercholesterolemia and artherosclerosis. Bioavailability of protein and mineral elements could be enhanced by reducing the phytic acid; low level of phytic acid in legumes is desirable because of the health benefit effects (Famularo *et al*., 2005).

The raw sample of *C. ensiformis* contains 5.29 mg/g saponin. All the hydrothermal processing methods had varying degrees of effects in reducing the level of saponin. After hydrothermal processing, the percentage range of reduction was 72.40 to 78.83. Boiling appeared to induce more reduction than steaming. Earlier study on *Albizzia lebbeck* showed reduction percentages of 12.54 and 56.00 after boiling

Table 4. Antinutritional components of *C. ensiformis* as influenced by hydrothermal processing methods (mg/g).

Antinutrtional components	Processing conditions				
	RS	BAP	SAP	BEP	SEP
Phytic acid	59.28±0.26^{c}	21.78±0.20^{a} {63.26}	25.48±0.53^{b} {57.02}	21.95±0.46^{a} {62.97}	25.84±0.61^{b} {56.41}
Saponin	5.29±0.33^{e}	1.12±0.01^{a} {78.83}	1.46±0.12^{d} {72.40}	1.27±0.02^{b} {75.99}	1.36±0.04^{c} {74.29}
Trypsininhibitor activity	25.61±0.21^{b}	0.00±0.00^{a} {100.00}	0.00±0.00^{a} {100.00}	0.00±0.00^{a} {100.00}	0.00±0.00^{a} {100.00}
Tannin	27.47±0.44^{d}	9.70±0.41^{a} {64.69}	10.60±0.34^{c} {61.41}	10.03±0.20^{b} {63.49}	10.64±0.32^{c} {61.27}

Values above are means ± standard deviation (n=3) on dry basis; means with different letters in the same row are significant (p<0.05). Values in parenthesis represent % decrease.
RS=raw dried sample; BAP=boiling at normal atmospheric pressure; SAP=steaming at normal atmospheric pressure; BEP=boiling at elevated pressure; SEP=steaming at elevated pressure.

Table 5. *In vitro* multienzyme protein digestibility of *Canavalia ensiformis* before and after hydrotherrmal processing.

Processing methods	10 min		15 min	
	pH	% Digestibility	pH	% Digestibility
RS	9.36	41.01±0.08^{a}	9.35	41.20±0.08^{a}
BAP	6.90	85.55±0.07^{d} {108.56}	6.89	85.73± 0.01^{d} {108.08}
SAP	6.92	85.19± 0.03^{c} {107.68}	6.90	85.55±0.06^{c} {107.65}
BEP	6.90	85.55±0.07^{d} {108.56}	6.89	85.73±0.03^{d} {108.08}
SEP	6.93	85.01±0.01^{b} {107.24}	6.92	85.50±0.08^{b} {106.77}

Values above are means ± standard deviation (n=3) on dry basis; means with different letters on the same column are significant (p<0.05). Values in parenthesis represent % increase in digestibility.
RS=raw dried sample; BAP=boiling at normal atmospheric pressure; SAP=steaming at normal atmospheric pressure; BEP=boiling at elevated pressure; SEP=steaming at elevated pressure.

for 30 min and 60 min, respectively (Abdullahi *et al.*, 2007). Reduction of saponin after hydrothermal processing could be due to the breakage in the linkage of the carbohydrate moiety from aglycone of steroid or triterperoids bound through glycosidic linkages (Machaiah *et al.*, 1999). Saponin are chelating agents, their presence at high concentration hinders availability of essential nutrients. Nonetheless, as in the case of phytic acid reports indicating dietary benefits of compounds such as saponin which are hitherto referred as antinutritional factors called for re-evaluation of these phytochemicals as antinutrients.

The TIA of the raw dried sample was 25.61 mg/g. After processing, all the four methods resulted in complete elimination of TIA i.e 100% reduction. These results agree with the findings on *Luffa aegyptiaca* in which domestic processing eliminated trypsin inhibitor (Elemo *et al.*, 1998). After boiling of *Prosopis africana* seeds for four hours, there was complete destruction of trypsin inhibitor (Odo *et al.*, 2004). Complete elimination of trypsin inhibitor during thermal processing might be due to the denaturation of the protein structure of the trypsin inhibitor. Trypsin inhibitor is a protease inhibitor which inhibits proteolytic enzymes by binding them in a one to one ratio thereby impairing protein hydrolysis.

Processing by boiling and steaming at normal atmospheric pressure resulted in reduction of 64.69 and 61.41% of tannin, respectively while at elevated pressure tannin reduced by 63.49 and 61.27%, respectively. In general, processing methods caused significant reduction (p<0.05) in the tannin content of the legume. Tannins are water soluble phenolic compounds (Siddhuraju *et al.,* 2002). Reduction of tannin during the hydrothermal processing may

be attributed to leaching out of the phenol into the cooking water. Tannin forms complexes with carbohydrates, protein and certain metal ions under favourable conditions of pH and concentration. Formation of tannin-protein complexes has been reported to be responsible for growth depression (Reddy *et al.*, 1995). Therefore, reduction of tannin in this legume studied should improve the nutritive value of dishes prepared from it.

In vitro *protein digestibility (IVPD) of the legume*

The need for *in vitro* protein digestibility studies arises since it has been reported that nutrients composition of foods is not adequate enough to determine nutrient bioavailability (Fagbemi, 2005; Ayo *et al*., 2007). Heat of the hydrothermal processing technique used in this study has significant effects (p<0.05) on the percentage protein digestibility. The IVPD of the raw sample was low --- 41.01% at 10 min. This was probably due to presence of high concentration of *in-situ* antinutritional components. Raw sample of soyabean and lima bean were reported to have percentage protein digestibility of 5.4% and 36.6%, respectively (Adewusi and Osuntogun, 1991). The IVPD increased significantly after hydrothermal processing. The highest IVPD of 85.73% was observed when the sample was boiled. Of all the processing methods, SEP resulted in the lowest IVPD of 85.50%. Processing methods such as natural fermentation and defatting have been reported to improve/increase IVPD (Oyebode *et al.*, 2007). Dry heat such as roasting reduced protein digestibility because of mallaird reaction involving interaction between protein and sugar causing irreversible formation of compounds thereby decreasing the quantity of protein present for digestion (Fagbemi, 2005; Ayo *et al.*, 2007).

Conclusion

Soaking and hydrothermal processing methods had significant effects on the nutrients, antinutrients as well as the in vitro protein digestibility of *C. ensiformis.* Soaking followed by hydrothermal processing methods could be used to alleviate the problem of prolonged cooking of underutilised legumes. Since the percentage protein digestibility of this underutilised hard-to-cook legume is high after hydrothermal processing, it is expedient to consider it for further food utilisation in local dishes such as *moin-moin, ekuru, akara* and others. Utilisation of this legume could foster economic utility, expand market value and prevent PEM by providing cheap protein that could meet the nutritional needs of the world population, particularly those of the developing nations.

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