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Effect of Alkaline Treatment on Nutrient and Antinutrient Contents of *Mucuna pruriens* (L.) DC.

Ebenezer Ola Falade[1*] and Anthony Okhonlaye Ojokoh[1*]

[1]Department of Microbiology, Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author EOF designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed the analyses of the study. Author AOO managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate the impact of Trona and woodash (alkaline tenderizers) on aniti-nutrient and nutrient content of *Mucuna pruriens* during fermentation.
Study Design: Natural fermentation and fermentation using additives (4% Trona, GWA-Gmelina wood ash) was implored.
Place and Duration of Study: Department of Microbiology, Federal University of Akure, Ondo State between November 2014 and March 2015
Methodology: Microbial analysis was carried out using Sabouraud dextrose agar, nutrient agar and De man Rogosa agar. pHand total titratable acidity analysis were carried out. Proximate, mineral composition, antinutrient analysis and *in-vitro* protein digestibility was also carried out on the fermented samples.
Results: A total of eighteen (18) microorganisms were isolated during fermentation; eleven (11) bacteria (*Bacillus subtilis*, *Bacillus licheniformis, Staphylococcus aureus, Micrococcus luteus, Pseudomonas aeruginosa, Arthrobacter* sp., *Lactobacillus plantarum, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus acidophilus, Lactococcu scremoris)*, two (2) yeast

**Corresponding author: E-mail: sugarrayebenson@gmail.com, tonyojokoh@yahoo.com;*

(*Saccharomyces cerevisiae, Geotrichum candidum)* and five (5) molds*, (Rhizopus stolonifer, Aspergillus flavus, Aspergillus niger, Fusarium oxysporium* and *Eurotium rubrum).* pH values obtained reduced with increase in fermentation period, Total Titratable acidity (TTA) increased with fermentation. The antinutrient content of the samples decreased significantly with fermentation. A total of 78%, 94%, 74% and 77% reductions in phenol, trypsin, phytate, and tannin were recorded respectively in trona fermented samples. On the other hand, GWA samples recorded most improvement in the proximate analysis results (ash-4.2%, moisture-12.36%, fat-17.63%, and protein-39.51% contents). Furthermore, the highest IVPD (*In-vitro* protein digestibility) was recorded after the beans were cooked and autoclaved but IVPD drop drastically by 27.6% with Trona fermentation. Results obtained indicate samples fermented with Trona (TR) recorded the highest reduction in antinutrient content of the beans. On the other hand, GWA samples recorded most improvement in the proximate analysis results (ash-4.2%, moisture-12.36%, fat-17.63%, and protein-39.51% contents). Furthermore, the highest IVPD (*In vitro* protein digestibility) was recorded after the beans were cooked and autoclaved but IVPD drop drastically by 27.6% with Trona fermentation.

Keywords: Fermentation; Trona; Gmelina wood ash; antinutrients; Mucuna pruriens.

1. INTRODUCTION

Mucuna pruriens are annual or perennial, herbaceous, vigorous climbing vines, with fleshy nodulated roots, slender long trailing stems and numerous trifoliate leaves on short, hairy petioles. The seed pods are covered with microscopic velvety hairs (called trichomes) that can be extremely painful if they get into your eyes or could course itchy blister when they come in contact with skin. They are generally bat pollinated and produce seeds that are buoyant sea beans. Pods are produced on long, rope-like stems that hand from the forest canopy [1]. The itching beans *Mucuna pruriens* is an underutilized legume species grown predominantly in Asia, Africa, in parts of America [2]. The bean is only promoted by smallholder farmers in Africa, South America and South Asia as a green manure or a cover crop [3,4]. However, the lack of knowledge of the nutritional qualities of lesser-known legumes grown in developing countries like Nigeria is responsible for the poor utilization of these traditional crops in different food formulations. Despite its nutritional potential: rich in protein (23%-35%) and its digestibility comparable to other pulses like soy bean, lima bean and rice bean [5,6] *Mucuna pruriens* remains a minor food crop. It is poorly adopted in agricultural systems [7] which arise from the presence of secondary metabolite in plants and include trypsin and chymotrypsin inhibitors, polyphenols, nicotine, phytostigmine, serotonin and phytates. Anti-nutritional compounds reduce food intake and nutrient utilization in animals and lower the nutrient value of grain legumes [8]. According to [9] the major anti-nutritional compound in *Mucuna pruriens* is a non-protein amino acid, 3, 4-dihydroxy-L-phenylalanine (L-Dopa). Increased serum level of L-Dopa from consumption of *Mucuna pruriens* leads to high concentration of dopamine in peripheral tissue inducing anti-physiological effects such as nausea, vomiting, anorexia, paranoid delusions, hallucinations, delirium, severe depression and unmasking dementia [10,11]. Since the average L-Dopa content in mucuna bean is 3.1 - 6.7% [8], therefore to effectively utilize the legume to its full potential as food, inactivation or removal of antinutritional factors by adopting economically viable processing techniques is required.

Fermentation is one of the processes that decreases the level of antinutrients in food grains and increases the starch digestibility, protein digestibility and nutritive value [12] The nutritional evaluation of fermented grains has been examined by many workers [13,14,15] Fermentation also leads to an increase in protein content [16] enhancement of carbohydrate accessibility, improvement in amino acid balance [17], decrease in antinutritional factors like tannin and phytic acid [18]. However, Pre-treatment of the bean before fermentation provided the most effective means of reducing L-Dopa in velvet bean [19]. According to [19] 90% (89.95%) of L-Dopa was reduced after the beans were boiled for 45 min, dehulled, soaked for 12 or 24 h with removal and replacement of water after 12 h, and then boiled in fresh water for an additional 45 min. Further reduction was obtained during fungal fermentation, suggesting the production of an L-Dopa-degrading enzyme. Other physical and biochemical methods used to process dry legumes include soaking, cooking, selective

filtration, irradiation, enzymatic treatments and germination.

In legume processing, where a method is not effective in removing antinutritional compounds, a combination of two or more methods is used [20]. *Mucuna pruriens* seeds contain other antinutritional compounds such as protease inhibitors, phenolic compounds and phytates.

It is therefore expected that combination of hydrothermal treatments and fermentation with alkaline tenderizers (e.gtrona or wood ash) will be effective processing methods of reducing the anti-nutrients of the seeds, reduce toxic effect and enhance the availability of essential amino acids.

2. METHODOLOGY

2.1 Collection of Samples

Mucuna pruriens were procured from International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State Nigeria. Trona was procured from Sabo market in Akure. Wood from *Gmelina arborea* was made into a fire to become ash completely and 2 kg of cooled and ground ash was collected.

2.2 Processing of *Mucuna pruriens*

The processing treatments used for the reduction/elimination of antinutrient content were Boiling, auto-claving, dehulling and fermentation. The seeds were divided into two portions. One portion received no heat treatment (Raw mucuna) while the other portion BM (Boiled mucuna) was cooked in distilled water (100℃) in the ratio of 1:10 (w/v) on a hot plate for 90 min according to Ukachukwu and Oboira [21]. The beans seed became soft and ready for dehulling.

2.2.1 Dehulling

Hulls were removed manually after boiling the seeds for 90 min according to El-Beltagy [22].

2.2.2 Autoclaving

After boiling, the water was decanted and the boiled beans were subjected to further heat treatment by autoclaving for 30 mins at a temperature 121℃ at 1 kgf/cm^2 according to Mugendi et al., [23].

2.3 Fermentation of *Mucuna pruriens*

As described by [24], Boiled *Mucuna pruriens* was sub-divided into 4 portions where one portion was left unfermented (BMN-Boiled Mucuna Non-fermented). Second portion of BM was subjected to Natural Fermentation, by soaking twenty (20) g of BM in 120 ml of distilled water and incubated in an incubator (Orbital shaking incubator, REMI/ 396LAG) at 37℃ in a 1:6 (bean: water) ratio for 72 h, and left to ferment naturally. The Third and Forth portion were subjected to spontaneous fermentation as described above with the addition of 4% Trona (lake salt which is largely hydrated sodium carbonate TR) and Gmelina Wood ash (GWA) respectively and fermented for 72 h. After processing, the water was decanted, and the beans dried at 60-70℃ for 20 h in hot oven, ground with a Wiley laboratory mill to pass through a 1-mm mesh, and preserved in airtight sample bottles for analyses.

2.4 Microbial Analysis

Samples were collected in triplicates at 24 h intervals during fermentation and serial dilution of the samples were carried out aseptically in test tubes by transfer of 1 ml of supernatants of samples into 9 ml of sterile peptone water in test tubes .The mixtures were thoroughly mixed diluted serially from 10^{-4} to 10^{-5} . A sterile wire loop was used to collect samples from the test tube and inoculated into the plate containing Sabouraud dextrose agar for fungi isolation and Nutrient Agar (NA) for bacterial isolation while De Man Rogosasharpe agar was used to isolate lactic acid bacteria. Nutrient agar plates were incubated at 32℃ for 18-24 h; Sabouraud dextrose agar plates were incubated at 24℃ for 48-72 h while De Man Rogosasharpe agar plates were incubated at 32℃ for 18-24 h anaerobically. Pure colonies obtained were preserved on media agar slants in McCartney bottles and stored in the refrigerator until required for further identification and characterization.

2.5 Determination of pH and Total Titratable Acidity

At each fermentation periods, pH was read using Hanna Instruments 8418 pH-meter. The titratable acidity was estimated by titrating against 0.1 M NaOH to phenolphthalein end-point and the acidity later calculated as lactic acid/100 g [25]. A 10 ml of fermenting broth was transferred into a

beaker and few drops of phenolphthalein indicator were added. This was titrated with 0.1M sodium hydroxide (NaOH) until an end point of permanent pink colour was reached while the initial and the final volume of the NaOH titrated were noted. This procedure was repeated two more times and the average volume of NaOH used was determined. Total titrtable acidity was expressed as percent (%) of lactic acid.

$$\% \text{ acid} = \frac{[\text{mls NAOH used}] \times [0.1\ \text{NaOH}] \times [\text{milliequivalent factor}] \times [100]}{\text{Grams of sample}}$$

2.6 Proximate Chemical Composition Analysis of Velvet Beans

All samples were analysed in triplicates for crude protein, moisture, crude fat, ash, crude fibre, tannins, phytate and minerals as chemical analysis followed methods of [26].

2.7 Mineral Content Analysis

The mineral content was determined using the method described by Adeyeye and Adewoke [27]. One gram of dried samples was digested with 25 ml of 0.03 N hydrochloric acid (HCl). The digest was boiled for 5 minutes, allowed to cool to room temperature and transferred to 50 ml volumetric flask where the volume was made up to the mark. The resulting digest was filtered with ashes What man No. 1 filter paper and ready for analysis. The filtrate was analyzed for mineral (calcium, iron, potassium and phosphorus) content using Atomic Absorption Spectrophotometer.

2.8 Anti-nutritional Factor Determination

Tannin was determined by the modified vanillin method of Price et al. [28]. Phytic acid in both raw and treated seed samples was determined according to the method of Mohamed et al. [29] using chromogenic solution. The amount of phytic acid content was expressed as mg/ g dry sample Trypsin inhibitor activity (TIA) was determined by the method of Kakade et al. [30]. Folin-Dennis method described by Pearson [31] was used to determine the phenol content.

2.9 *In vitro* Protein Digestibility (IVPD)

The *in-vitro* protein digestibility of each sample was evaluated using a sequential pepsin and pancreatin digestion model according to the method of [32]. A 1g of the sample was suspended in 60 mL of 0.1 M HCL at pH of 1.0 containing 6 mg of pepsin, followed by gentle shaking for 15 min at 37℃. The resulting solution was neutralized with 0.5 M NaOH to 7.0 and treated with 16 mg of pancreatin in 30 mL of 0.1 M phosphate buffer (pH 8.0). The mixture was then shaken for 24 hours at 37℃ in a water bath. The undigested solid was separated by filtration. The protein content of the undigested solid and initial protein count of the sample was determined using the Kjeldahl method [33]. *In-vitro* protein digestibility was expressed as percentage as indicated below:

$$\textit{In-vitro} \text{ protein digestibility (\%)} = \frac{A-B}{B}$$

Where

A= % protein in the samples before digestion
B= % protein after enzyme digestion

2.10 Statistical Analysis

All data obtained were carried out in triplicates and subjected to descriptive statistics, analysis of variance (ANOVA) and Duncan Multiple Range Test and the level of significance was set at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 Microorganisms Isolated during the Fermentation of *Mucuna pruriens*

Eighteen (18) microorganisms were isolated during *Mucuna pruriens* fermentation from which eleven (11) were bacteria, five (5) were moulds and two (2) were yeast. These are *Bacillus subtilis*, *Bacillus licheniformis, Staphylococcus aureus, Micrococcus luteus, Pseudomonas aeruginosa, Arthrobacter* sp., *Lactobacillus plantarum, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus acidophilus, Lactococcus rubrum, Rhizopus stolonifer, Saccharomyces cerevisiae, Geotrichum candidum, Lactobacillus cremoris, Aspergillus flavus, Aspergillus niger, Fusarium oxysporium, Eurotium rubrum* and *Staphylococcus aureus*

3.2 Changes in the Bacterial Population during Fermentation of *Mucuna pruriens*

Fig. 1 shows the bacterial count of fermented *Mucuna pruriens* samples. The initial bacterial count of all NF, TR and GWA were 4.93×10^8,

2.45× 10^8, and 4.73× 10^8 cfu/ml respectively. Bacterial Count of natural fermented samples increased to 7.23 while a tremedious increase was recorded in *Gmelina* fermented samples from 4.73× 10^8 to 8.73× 10^8 cfu/ml. GWA, Trona fermentation recorded the least count of bacteria while the bacterial count of NF increased to 7.35 ×10^8 after 48 hours and reduced slightly to 7.23 ×10^8 after 72 h.

3.3 Changes in Fungal Population during Fermentation of *Mucuna pruriens*

As shown in Fig. 3 that there was no fungal growth recorded at 0h for all fermentation types carried out. However, NF recorded high growth of fungi from 1.72×10^5 sfu/g to 4.2×10^5 sfu/gwhile TR had no fungal growth after until 48 hrs (0.75×10^5 sfu/g). GWA had an initial growth of 1.21×10^5 sfu/g at 24 hr and increased to 2.45×10^5 sfu/g at 72 hrs.

3.4 Microbial Occurrence during Fermentation

Table 1 and 2 shows the occurrence and succession of different microorganisms during fermentation periods. *Bacillus subtilis* was isolated from all samples of *Mucuna pruriens* including the boiled samples. Similarly, *Bacillus licheniformis* was as well isolated from all samples except boiled samples. *Micrococcus luteus* and *Arthrobacter* sp. were only isolated from NF (natural fermented) samples at 0-24 h while *Pseudomona* ssp occurred in both NF and GWA samples. Finally, all eleven bacteria isolated in this study occurred in Naturally Fermented (NF) samples including the lactic acid bacteria. Among all lactobacillus isolated, *Lactobacillus casie* was commonly isolated from NF, TR and GWA samples between 48-72 h.

However, fungal isolation was common on NF samples as it is presented in the above mentioned table. *Aspergillus flavus* and *Saccharomyces cerevisiae* were isolated from all fermented samples after 24 h from NF and GWA (*Gmelina* ash fermented) samples and was also isolated after 72 h from TR (Trona Fermented) samples.

3.5 Changes in pH during Fermentation of *Mucuna pruriens*

Fig. 3 represents the pH values of fermented samples. The pH results of NF samples reduced from 6.98±0.01 at 0 h to 4.70±0.02 after 72 h. TR fermented sample had a drastic reduction in pH from 8.40±0.01 to 6.56±0.01 within 0-72 h. The same trend was observed during wood ash fermentation (GWA) where pH decreased from 7.80±0.01 to 6.42±0.02 at 0 h to 72 h.

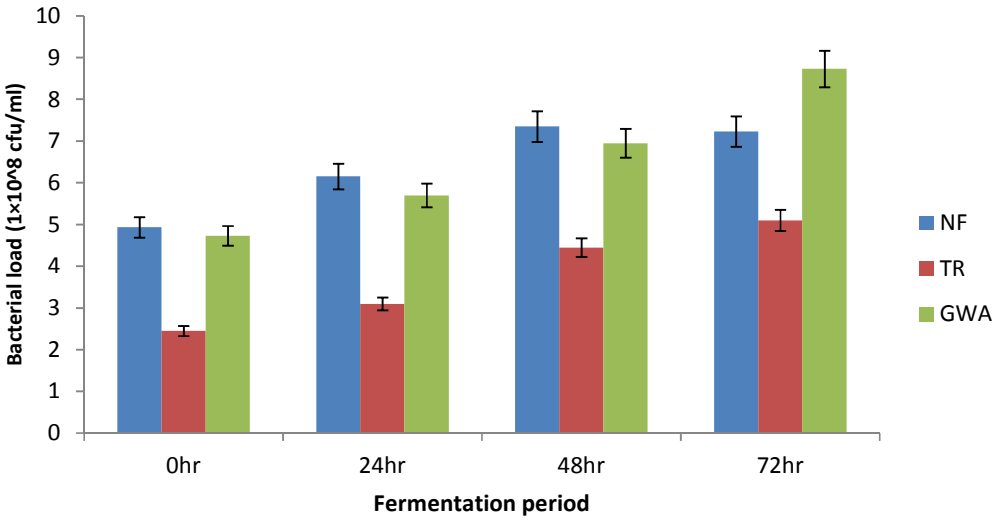

Fig. 1. Bacterial count of fermented *Mucuna pruriens* samples

Keys: NF-Naturally fermented Mucuna pruriens, TR-Trona fermented Mucuna pruriens, GWA- Mucuna pruriens fermented with Gmelina wood ash

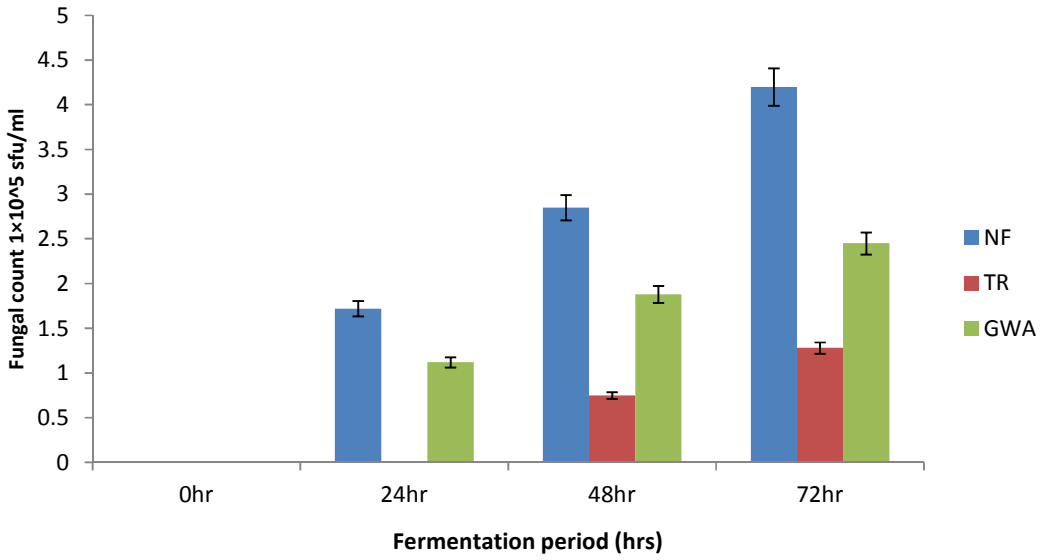

Fig. 2. Fungal count of fermented *Mucuna pruriens* samples
Keys: NF- Natural fermented Mucuna pruriens,
TR-Trona fermented Mucuna pruriens,
GWA- Mucuna pruriens fermented with Gmelina wood ash

Table 1. Occurrence of microorganisms isolated from processed and fermented *Mucuna pruriens*

Isolates	RM	BM	NF	TR	GWA
Bacteria					
Bacillus subtilis	+	+	+	+	+
Bacillus licheniformis	+	-	+	+	+
Staphylococcus aureus	+	-	+	-	-
Micrococcus luteus	-	-	+	-	-
Pseudomonas aeruginosa	-	-	+	-	+
Arthrobacter sp.	-	-	+	-	-
Lactobacillus plantarum	-	-	+	-	-
Lactobacillus casei	-	-	+	+	+
Lactobacillus fermentum	-	-	+	-	+
Lactobacillus acidophilus	-	-	+	+	+
Lactococcus cremoris	-	-	+	-	+
Fungi					
Aspergillus flavus	+	-	+	+	+
Aspergillus niger	-	-	+	-	+
Fusarium oxysporium	+	-	+	-	+
Eurotium rubrum	-	-	+	-	-
Rhizopus stolonifer	+	-	+	+	+
Geotrichum candidum	+	-	+	+	+
Saccharomyces cerevisiae	+	-	+	+	+

Key: RM- Raw mucuna, BM-Boiled mucuna, NF-Naturally fermented mucuna, TR-Trona fermented Mucuna, GWA- mucuna fermented with Gmelina wood ash
+: Positive- : Negative

Table 2. Microbial succession during fermentation of *Mucuna pruriens*

Samples	Time of fermentation (hrs)			
	0	**24**	**48**	**72**
NF	*Staphylococcus aureus, Bacillus subtilis , Micrococcus luteus, Bacillus licheniformis*	*Pseudomonas aerugiosa, Athrobacter* sp., *Eurotium rubrum, Aspergillus niger, Aspergillus flavus*	*Bacillus subtilis, Lactobacillus casei, Lactobacillus fermentum, Fusarium oxysporium, Rhizopus stolonifer.*	*Lactobacillus acidophilus, Lactococcus cremoris, Saccharomyces cerevisiae, Geotrichum candidum*
TR	*Bacillus subtilis, Bacillus licheniformis*	*Aspergillus niger, Saccharomyces cerevisiae*	*Lactobacillus casei Aspergillus niger, Aspergillus flavus*	*Lactobacillus acidophilus, Rhizopus stolonifer, Saccharomyces cerevisiae, Geotrichum candidum*
GWA	*Bacillus licheniformis, Pseudomonas aeruginosa,*	*Bacillus subtilis, Aspergillus flavus, Aspergillus niger*	*Lactobacillus casei, Geotrichum candidum Fusarium oxysporium, Saccharomyces cerevisiae.*	*Lactobacillus fermentum, Rhizopus stolonifer,*

Keys: NR- Natural fermented samples,
TR- Trona fermented samples,
GWA- Gmelina Wood Ash fermented samples

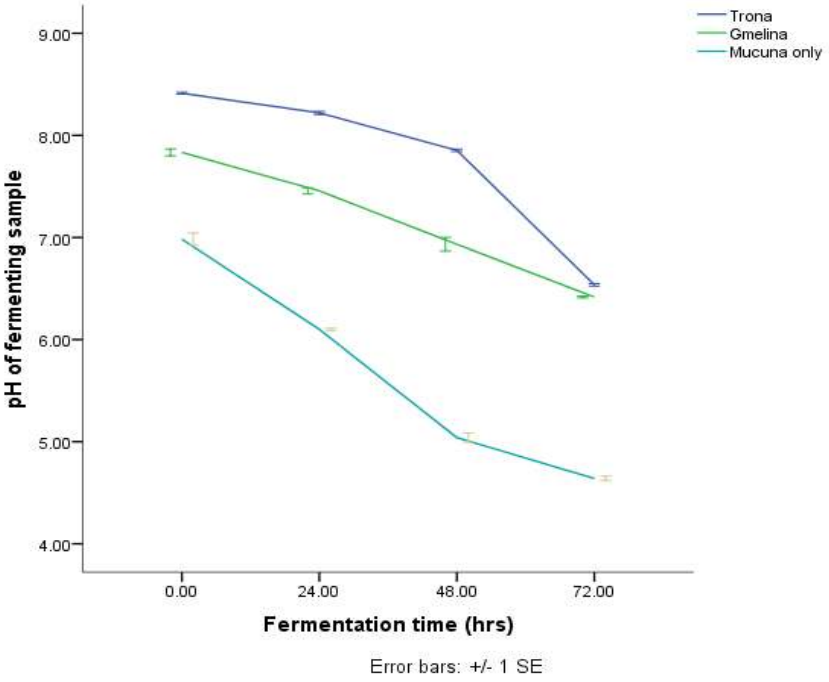

Fig. 3. Changes in pH during fermentation of *Mucuna pruriens* samples

3.6 Changes in Total Titratable Acidity (TTA) during Fermentation of *Mucuna pruriens* Samples

Variations in total titratable acidity (TTA) during fermentation of *Mucuna pruriens* are shown in Fig. 4. While pH values obtained reduced with increase in fermentation period, Total titratable acidity (TTA) increased and maximum TTA recorded was 1.9% in NF samples. TR fermented sample had an initial TTA of 0.10±0.01 at 0 hour which increased to

0.46±0.01 at 24 h, reduced to 0.25 at 48 h and finally increased to 0.60±0.02 at 72 h. GWA fermented samples increased from 0.62±0.02 at 24 h to 1.20±0.01 at 72 h.

3.7 Changes in Proximate Composition of *Mucuna pruriens* Samples

The result of proximate composition of treated mucuna samples are shown in Table 3. GWA samples recorded most improvement in the proximate analysis results: (moisture-12.36%, fat-17.63%, ash -4.2% and protein-39.51% contents). Boiled mucuna samples (BM) had the lowest moisture content (8.82±0.01%) while GWA exhibited highest moisture content of 12.39±0.02%.

It was also confirmed in this study that both boiling and fermentation increases protein content of the beans. Boiled samples contain 15.96±0.02 fat, naturally fermented, Trona fermented, and Gmelina wood ash fermented samples had increased fat content of 16.351±0.02, 16.915±0.01 and 17.629±0.04 respectively.

This research also reveals that Raw Mucuna samples (RM) are low in protein content (27.52±0.03%) and even lower after boiling and autoclaving with 5.62%. Nevertheless, tremendous increase was observed after fermentation. Trona fermented samples had the highest protein content of 39.52±0.01.

3.8 Antinutrient Composition of *Mucuna pruriens* Samples

The changes in antinutrients composition of the samples are shown in Figs. 5 and 6. The results obtained from anti-nutrient composition of treated samples indicate samples fermented with Trona (TR) recorded the highest reduction in antinutrient contents of the beans. A reduction of 94%, 74.2%, 77.2% and 29.3% in trypsin inhibitor, phytate, Tanins and phenols were recorded respectively in TR (Trona fermented samples).

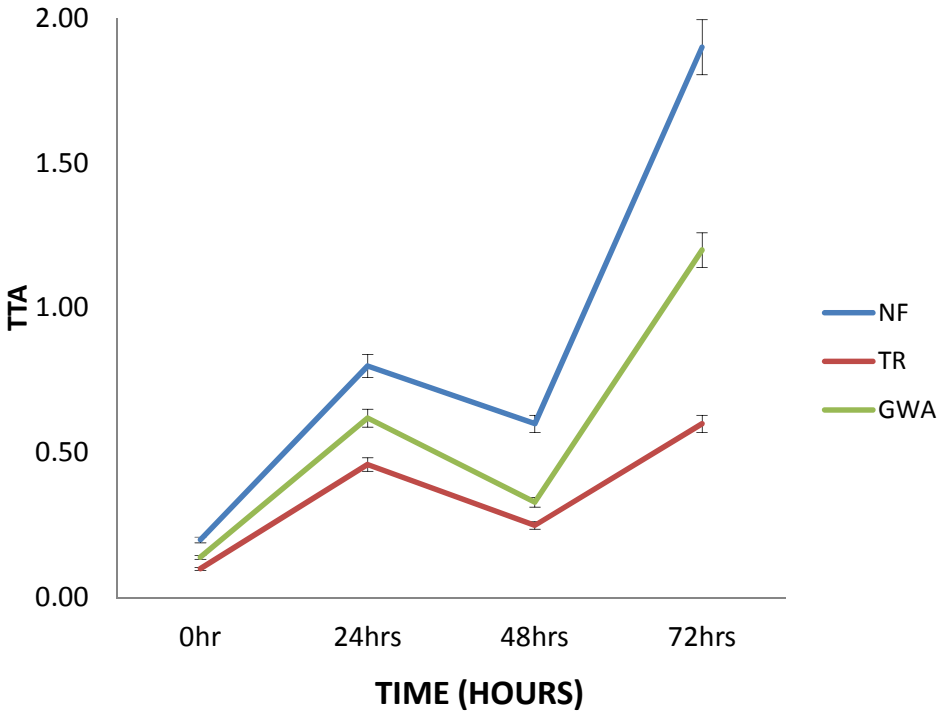

Fig. 4. Total titratable acidity (TTA) variations during fermentation of *Mucuna pruriens* samples

Legend: RM- Raw mucuna, BM-Boiled mucuna, NF-Natural fermented mucuna, TR-Trona fermented Mucuna, GWA- mucuna fermented with Gmelina wood ash

Table 3. Proximate (%) composition of treated *Mucuna pruriens* samples

Samples	Proximate composition					
	Ash	Moisture	Fat	Fibre	Protein	CHO
RM	4.10±0.02^{e}	9.83±01^{b}	3.10±0.01^{a}	9.76±0.01^{d}	27.57±0.03^{b}	35.83±0.09^{c}
BM	2.86±0.01^{d}	8.82±0.01^{a}	15.96±0.02^{c}	0.37±0.01^{a}	5.64±0.02^{a}	66.40±0.01^{e}
NF	1.13±0.12^{a}	11.16±0.01^{c}	16.39±0.02^{d}	0.48±0.01^{b}	37.32±0.01^{d}	33.87±0.02^{b}
TR	2.57±0.02^{c}	11.72±0.01^{d}	17.11±0.04^{e}	0.37±0.01^{a}	39.52±0.01^{e}	35.42±0.02^{d}
GWA	2.22±0.01^{b}	12.39±0.02^{e}	12.38±0.01^{b}	0.73±0.01^{c}	33.12±0.01^{c}	27.63±0.02^{a}

Legend: RM- Raw mucuna, BM-Boiled mucuna, NF-Natural fermented mucuna, TR-Trona fermented Mucuna, GWA- mucuna fermented with Gmelina wood ash, CHO-Carbohydrate

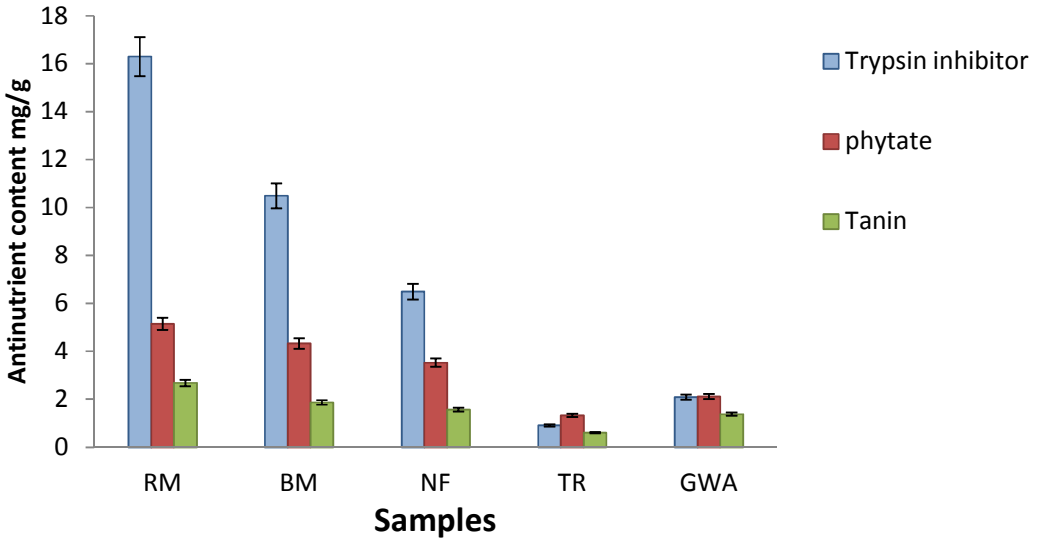

Fig. 5. Antinutrients content of *Mucuna pruriens* samples

Legend: RM- Raw mucuna, BM-Boiled mucuna, NF-Natural fermented mucuna, TR-Trona fermented Mucuna, GWA- mucuna fermented with Gmelina wood ash

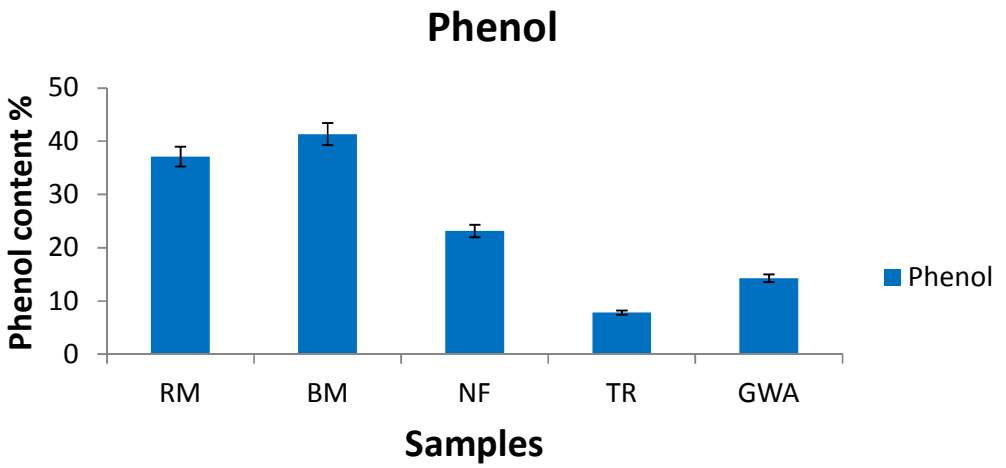

Fig. 6. Phenol content of treated *Mucuna pruriens* samples

Legend: RM- Raw mucuna, BM-Boiled mucuna, NF-Natural fermented mucuna, TR-Trona fermented Mucuna, GWA- mucuna fermented with Gmelina wood ash

3.9 Mineral Composition of *Mucuna pruriens* Samples

The changes in mineral content of mucuna samples are represented in Table 4. Natural fermented samples (NF) had the lowest sodium content with value 433.67±0.33. Raw mucuna samples (RM) had the highest sodium content with value of 584.33±0.88. The sodium content of TR and GWA samples are 556.33±0.08 and 433.67±0.03 respectively.

Potassium content was highest in Boiled *Mucuna* (BM) samples (296.33±0.33) while samplesfermented with Gmelina woodash (GWA) exhibited the lowest potassium content of 164.00±0.58. There was no significant difference (P≤0.05) between raw mucuna (RM- 68.09±0.01) samples and boiled Mucuna samples (BM-68.02±0.01). RM samples had highest magnesium content recorded (98.03±0.01) while natural fermented samples (NF) recorded 48.04±0.01 which was the lowest magnesium recorded in this study.

Finally, there was significant difference (P≤0.05) between all fermented samples. However, BM and TR samples had no significant difference (P≤.05). BM had the highest iron content with value of 4.11±0.01.

3.10 Changes in *In-vitro* Protein Digestibility of *Mucuna pruriens* Samples

It is important to point out from the *in-vitro* protein digestibility result that highest IVPD (*In vitro* protein digestibility) 82.06% was recorded after the beans were cooked and autoclaved but IVPD drop significantly (P≤0.05) to the least when fermented with trona. Details of IVPD results are shown in Fig. 7.

Table 4. Mineral composition of *Mucuna pruriens* samples

Sample	Minerals				
	Sodium	Potasium	Calcium	Magnesium	Iron
RM	584.33±0.88^{e}	291.67±0.33^{d}	68.09±0.01^{c}	98.03±0.01^{e}	3.86±0.03^{c}
BM	565.00±0.10^{c}	296.33±0.33^{e}	68.02±0.01^{c}	95.33±0.03^{b}	4.11±0.01^{d}
NF	335.67±0.33^{a}	187.33±0.33^{b}	53.67±0.88^{a}	48.04±0.01^{a}	1.11±0.01^{a}
TR	556.33±0.88^{c}	259.67±0.88^{c}	68.91±0.01^{c}	82.03±0.01^{c}	4.09±0.01^{d}
GWA	433.67±0.33^{b}	164.00±0.58^{a}	56.02±0.01^{b}	65.73±0.03^{b}	1.73±0.01^{b}

Data are presented as Mean ±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05).

Legend: RM- Raw mucuna, BM-Boiled mucuna, NF-Natural fermented mucuna, TR-Trona fermented Mucuna, GWA- mucuna fermented with Gmelina wood ash

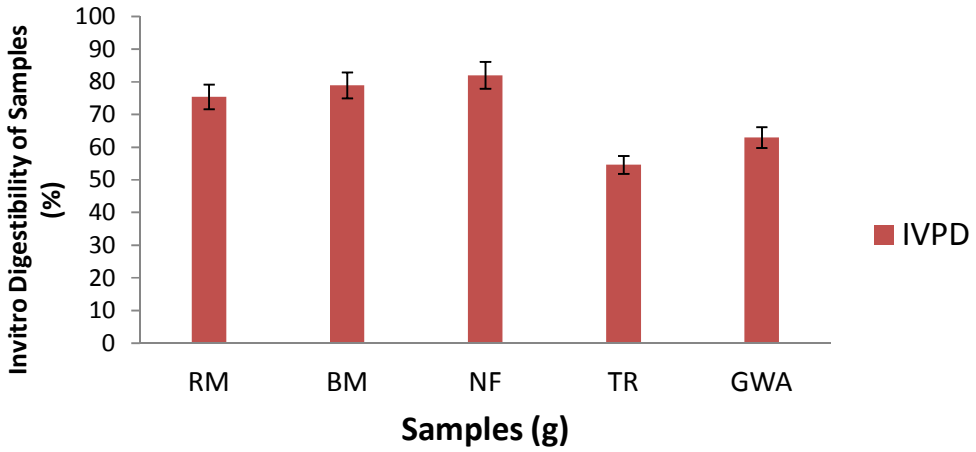

Fig. 7. *In vitro* Protein digestibility of *Mucuna pruriens* samples

Legend: RM- Raw mucuna, BM-Boiled mucuna, NF-Natural fermented mucuna, TR-Trona fermented Mucuna, GWA- mucuna fermented with Gmelina wood ash

4. DISCUSSION

Fermentation was found to cause a gradual reduction in pH with time. The change in pH from zero to 72 h resulted in a pH drop from 6.94 to 4.84 for NF (Natural fermentation) samples and from 7.90 to 6.71 for GWA (Gmelina Wood Ash fermentation). This result is in agreement to that of [34], who reported that, as a result of fermentation, acidity increased and pH falls down and that this inhibits microbial growth and also contributes to the flavour of processed millet. This result is also in agreement with [35] where they reported that Lactic Acid Bacteria fermentation causes a drop in pH of various food grains.

Concomitant with the drop in pH, there was a rise in TTA of fermented *Mucuna pruriens* throughout the fermentation process. For samples fermented without additive, the TTA increased from 0.2 to 1.90 after 72 h while the least TTA recorded was with samples fermented with Trona (0.6 after 72 h). The latter succession of yeast and LAB towards the end of fermentation period could explain the apparent increase in lactic acid contributing to a rise in acidity of the fermenting medium.

In vitro protein digestibility (IVPD) increased to maximum only with samples fermented without alkaline additive (NF). A 3.87% comparative increase in IVPD was recorded after boiled samples were fermented naturally. According to research conducted by [36], microflora may produce proteolytic enzymes during fermentation which may be responsible for the increased protein digestibility among all fermented samples. In addition, the elimination of phytic acid may contribute to the improvement in IVPD of fermented mucuna samples since fermentation has been shown to be more effective in reducing phytate. [37] had reported that the reduction in pH during fermentation plays an important role in enhancing native proteolytic enzymes activity and consequently promotes the breakdown of proteins to smaller polypeptides which are easily digested by enzymes.

Results obtained from the proximate composition indicates that all fermentation types increased the moisture content of mucuna where samples fermented with wood ash recorded the highest moisture content 12.39%. The significant increase in moisture content may be attributed to the fact that fermented samples spent longer time in water for 72 h [1].

Furthermore, a 79.54% and 96% reduction in protein and fibre respectively were recorded after raw samples were boiled. On the contrary, trona fermented samples were observed to increase in protein content by 43.34%. This increase could be attributed to the hydrolysis of the protein to amino acids and breakdown of tannin protein complex due to the addition of Trona [38]

The ash content of raw *Mucuna pruriens* was 4.10% which later decreased to 2.86% after boiling. This result is in accordance with the results of [39] who recorded decrease in ash content of pigeon pea seeds, from 5.50% (raw seeds) to 4.0% (boiled seeds). These loses could be as a result of leaching of the minerals into the boiling water. Also water absorption during boiling may cause dilution and hereafter, low amount of ash [40]. The observed decrease in ash content after cooking implies that the potential ability of these beans to supply essential minerals has been reduced. Furthermore, leaching of soluble salts into processing water may be the reason for slight reduction of ash during fermentation [41].

Lipids are distinct and diverse set of small molecules consisting of eight general compound classes which include: Fatty acids, Glycerolipids, Sterol lipids, phenol lipids e.t.c. The lipid content of RM (3.10%) is quite reasonable as all legumes contain reasonably moderate amount of lipid content. Nonetheless, subsequent treatment including boiling and fermentation had a positive increment in lipid content of the beans. The release of bound fat in cells of beans seed may be the major cause of fat increase after boiling and addition of alkaline tenderizer [42].

In this study, cooking and autoclaving had the only significant ($P \leq 0.05$) increase in starch content of the beans from 35.83% to 66.40%. This result is in agreement with [43] and also in agreement with [42]. The increase may be attributed to the breakdown of complex polysaccharides by action of heat which was otherwise bound in the raw sample.

Significant increase in iron (6.5%), potassium (1.6%) and calcium (2.92%) content was noticed during thermal treatment of *Mucuna pruriens*. Likewise, a 6.0% increase in iron was recorded in trona fermented samples. On the contrary, sodium and magnesium content got reduced after cooking and dehulling. Removal of seed coats led to reduction of some of the minerals which later got increased after addition of trona and woodash.

Results obtained from this study also confirmed that combination of heat and fermentation with trona brought major significant decrease in phytic acid. A 74% decrease in phytate was noticed after boiled *Mucuna pruriens* were fermented with trona. This may be partly due to leaching into the cooking medium, degradation by heat or formation of insoluble complexes between phytate and trona compound [44]. In addition, [45] had stated in his research that fermentation is known to cause a greater reduction in phytic acid to the low pH of fermented product, which is considered as optimum for phytase activity.

Tanins have been claimed to adversely affect protein digestibility [20,46,10] have reported that most tannin is located in the seed coat with only traces in the cotyledons. Since the seed coats are usually removed by soaking prior to consumption, the tannins in *Mucuna pruriens* are of little significance from the nutritional point of view. Besides, [47] and [48], also reported that in *Mucuna pruriens,* the levels of phenolics and tannins are reduced significantly during dry and wet heat treatments and their reduction improves the protein digestibility of the beans. This report support the result obtained in this study. A 30% reduction in tannin content was observed after boiling and autoclaving (i.e. 2.68 mg/g-1.87 mg/g). Further reduction was noticed after fermentation. Tannin content was totally eliminated to the minimum of 0.61 mg/g after trona fermentation.

In addition, heavy plunge in phenol content was recorded during fermentation. A great reduction of 81.1% and 65.5% were observed after cooked beans were fermented with Trona and Gmelina wood ash respectively. Trypsin inhibitor in diets leads to formation of irreversible enzyme-trypsin complexes. This causes a decrease in trypsin in the intestine and subsequently indigestibility of dietary protein, thus leading to slower animal growth [6]. In this study, trypsin inhibitor recorded from raw *Mucuna pruriens* (16.30%) was higher than the range reported by [49] i.e. 13.7-14.2%. Although, all methods applied in this study proved effective in reducing trypsin inhibitor content of *Mucuna pruriens*, however, the use of Tronain fermenting medium eliminated the antinutrient to the barest minimum of 0.91%. Same similar result was reported by [50] in Trona processed breadfruits but the mechanism behind this not yet known but it could be that Trona being a catalyst had aided in the breaking down the cell wall causing leaching thereby facilitates the reduction of the antinutrient [40].

Bacterial counts were observed to increase as the fermentation progressed in all the fermented *Mucuna pruriens*. Total viable counts and fungal counts increased with time in all the fermentation types. These results of these bacterial counts agree with [51] while studying the biochemical and microbial qualities of raw, boiled and fermented *Mucuna pruriens.* The most frequently isolated microorganisms from all the samples include B*acillus subtilis, Bacillus licheniformis, Aspergillus flavus, Rhizopus stolonifer*, and yeast. Many of these microorganisms have been isolated from legumes. Similar microorganism were isolated by [52] while studying the biochemical and microbial qualities of raw, boiled and fermented *Mucuna pruriens* (velvet bean). *Bacillus subtilis*and *Bacillus pumilus* have been confirmed among the main microorganisms responsible for the fermentation ofseed legumes. [53] isolated *Bacillus subtilis, Bacillus pumulis* and *Bacillus licheniformis* from tayohounta, a fermented baobab food from Republic of Benin. The sources of these microorganisms could be from raw materials, the environment and the processing equipment [54].

Furthermore, it is important point out that a typical succession of yeast and Lactic acid bacteria were noticed in all fermentation types used in this study. Yeast population in NF increased from 1.72×10^5 CFU/ml at 0h to 5.4×10^5 CFU/ml after 72h. Similarly, yeast count in GWA increased to the maximum of 4.06×10^5 CFU/ml after 72 h. The predominance of lactic acid bacteria which was followed by yeasts in this finding agrees with the reports of [55] and [56]. The presence of yeasts particularly *Sacharomyces cerevisiae* might lead to degradation of starch making them available for the lactic acid bacteria in an utilisable form thereby enhancing their rapid growth throughout the period. Moulds which were isolated at the initial phase of this research were in agreement with [50,55] also isolated *R. stolonifer, Aspergillus niger* and *Mucor mucedo* at the steeping phase of ogi production [57]. Similarly reported the presence of *Penicillium* and *Aspergillus* in fermenting maize dough during kenkey production which drastically reduced from 105 to less 102 cfu/g within 24 h of fermentation. Moulds have been reported to be surface flora of many grains. The major role of the moulds had been attributed to the saccharification of the carbohydrate content of the grains [58,56].

5. CONCLUSION

This research exercise has shown that all nutrient content of *Mucuna pruriens* analyzed improved significantly upon the use of economically viable methods (i.e. thermal & biochemical) applied. With regard to this, direct effect of pretreatment applied (boiling, dehulling and autoclaving) before fermentation (i.e. were noticeable). All antinutrients analyzed were reduced and more importantly, carbohydrate, potassium, calcium and iron content improved during thermal treatment.

Although all methods applied in this study proved to be effective in reducing antinutrient content, yet it was clearly established that alkaline additives (Trona and Woodash) indicated greatest potential in reducing antinutrient content of *Mucuna pruriens*. Specifically, the use of Trona to ferment *Mucuna pruriens* showed the greatest potential against the antinutrient analyzed. A total of 78%, 94%, 74% and 77% reduction in phenol, trypsin, phytate, and tannin was recorded respectively in trona fermented samples. Despite the potentials of trona stated above, caution must be applied in its use in processing food as it can be inferred from this research that trona exhibited an anti-digestibility upshot as it reduced the *in vitro* protein digestibility of *Mucuna pruriens*. Although some studies have investigated the biochemical and physiological effects of Trona [59,60] there is still a paucity of data concerning its dietary effects in humans.

Finally, woodash- a quintessence alkaline tenderizer also proved effective against antinutrients in this research. A noticeable fit was a significant increase in protein content and high *in vitro* protein compared to that of trona fermented samples. It can therefore be concluded that combination of hydrothermal treatment and fermentation with alkaline tenderizers (trona and woodash) are effective economically viable methods capable of reducing toxic/antinutrients of *Mucuna pruriens* and enhancing the nutrient availability of the beans.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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