Original Research Article

Effect of citric acid treatment and fermentation on the chemical composition of African yam bean (*Sphenostyli sstenocarpa*) and sensory evaluation of its gruel

Abstract

Aim:

Legumes, (particularly indigenous/ underutilized legumes), have been found to make substantial contributions to nutrient intakes of population groups especially in low resources environments. The African yam bean (AYB) is one of such indigenous legumes but has the problem of hard-to-cook phenomenon, beany flavour, bitter taste and some anti nutritional factors that limit its utilization. The aim of this study was therefore to determine effect of citric acid treatment and fermentation on the chemical composition of African yam bean (*Sphenostyli sstenocarpa*) and sensory evaluation of its gruel

Methodology: Cream coloured AYB seeds was purchased in Enugu, Enugu State Nigeria in the month of December. Sorted AYB was washed and fermented in citric acid medium (0.25%, 0.5% and 1%) for 24hrs, 48hrs and 72hrs at room temperature (28^{o}C) in a seed water ratio of 1:4 (w/v). The control was fermented without citric acid for 24hrs, 48hrs and 72hrs. At the end of the fermentation, each batch of the fermented seeds were divided into two. Half were dehulled and the other half were left whole. The fermented seeds were separately dried and milled into flour for further use. Standard laboratory methods were used for proximate, mineral and antinutrient analysis. Gruels were produced from all the flours and subjected to sensory evaluations using the nine-point hedonic scale. Data generated were analyzed using an IBM Statistical Package for Social Sciences (SPSS) database version 20.0. The analysis of variance (ANOVA) was used to compute the mean and standard deviations. Means were compared using the least significant difference (LSD) and significance accepted at $p<0.05$

Results: Chemical compositions of the flour from seeds of AYB fermented in 0.5% citric acid solution showed that protein contents increased by 50%, dietary fibre decreased by 0.02%. Raffinose, stachyose, lectins, trypsin inhibitors, tannins, oxalates, phytates and saponins were all significantly reduced to safe levels. Gruels made from whole and dehulled AYB fermented for 24hrs with 0.5% citric acid had significantly ($P< 0.05$) higher scores for aroma (7.30 & 7.35, respectively) and general acceptability (7.32 & 7.22 respectively). Dehulled AYB fermented for 24hrs in 1% citric acid had the best score (7.99) for colour. Based on the sensory evaluation result, gruels made from the AYB fermented in 0.5% citric acid was compared with the gruels made from AYB that was fermented in tap water and the result showed that gruels made from the AYB that was fermented in 0.5% citric acid for 24hrs had significantly better score for aroma (7.70), colour (7.10), and general acceptability (7.52) Food use of AYB can be improved by fermenting in 0.5% citric acid solution for 24hrs.

Keyword: citric acid, fermentation, proximate, anti-nutrient, sensory evaluation, gruel.

1. Introduction

Food and nutrition insecurity, which arises from limited access to both qualitative and quantitative food to meet dietary needs and food preferences are recurrent problems in Nigeria [1]. The food and nutrition insecurity situation analysis conducted in March 2018 by the United Nation (UN) agencies in Nigeria revealed that about 3.7 million Nigerians are facing food insecurity [2]. About 1 million Nigerians are in emergency food situation and immediate intervention might be needed [3]. Food utilization has been cited

as one of the causes of food and nutrition insecurity. It is not enough to have access to food, but the food should be free of secondary metabolites that antagonize their absorption and utilization. In achieving good nutrition and sustainable food security, the use of indigenous raw food materials has been proposed [4]. This is because knowledge and use of indigenous foods can help improve nutritional status and add variety to diets [5]. Eating right is essential to achieving good health. The knowledge of the best way to process and prepare food is essential to achieving proper utilization of the nutrients in food and consequently good health and nutrition.

Among the many underutilized indigenous foods in Nigeria, the Africa Yam bean (AYB) (*Sphenostylis stenocarpa*) has been cited as one of the crops that have the potential of meeting food and nutrition security in a sustainable way because of its great potentials [6]. It is a legume grown primarily for its dry seeds and is widely grown in most parts of Africa [7]. It is fried and eaten mostly as snacks or boiled and eaten as pottage or made into puddings in the eastern parts of Nigeria. Besides its use as food, it is also used for medicinal purposes. Studies have shown the potential of AYB in the prevention and management of Diabetes mellitus [8,9] and blood pressure with no negative effect in the liver [10]. Nutritionally, AYB rates higher than most legumes in terms of amino acid profile [11]. The protein concentrate is reportedly used for the fortification of starchy foods; but despite all these attributes, its use is limited by beany flavor and bitter taste, long cooking time of about 6 hours and flatulence inducing oligosaccharides [12, 13]

In this study, value has been added to AYB using different concentrations of citric acid to produce a wholesome flour free of beany flavour and bitter taste. The use of the flour to produce gruel have solved the problem of longing cooking time of about 6hours and reduced it to 5minutes.

2. Materials and methods

2.1 Cream coloured AYB seeds were purchased from Enugu in Enugu State, Nigeria. The seeds were sorted to remove impurities and weighed. The sorted AYB (20kg) was washed and fermented in citric acid medium (0.25%, 0.5% and 1%) for 24hrs, 48hrs and 72hrs at room temperature (28^{o}C) in a ratio of 1:4 (w/v). At the end of fermentation, AYB seeds were washed, each batch divided into two and one-half of the fermented portions dehulled, while the remaining were left whole. They were all placed in separate compartments in a digital food dehydrator which works by circulating cool dry air and removing moisture through a vent and ensuring food nutrients are not denatured (40-70 °C). A start up temperature was set at 70°C for 4hrs to dry up excess water and prevent further fermentation, then the temperature was reduced to 40°C for 44hrs when constant weight was obtained. They were milled into fine flour (70mm mesh screen) and stored in airtight containers and refrigerated until needed for chemical analysis and product development.

2.2 Proximate analysis

The methods described by the Association of Official Analytical Chemist in 2012 [14] were used in determining moisture, ash, total fat and crude protein content of the samples. Dietary fibre was carried out by Prosky, Asp, Furda, DeVries, Schweizer and Harland [15] method as described by AOAC Method 985.29. Carbohydrate was determined by difference [14].

2.2.1 Determination of moisture content

Two grams (2g) of the sample was weighed into a previously weighed crucible. The crucible plus the sample was taken and transferred into the oven set at 100ºC to dry to constant weight for 24 hours. The crucible plus the sample was removed from the oven, cooled for 10 minutes and reweighed. The sample in the crucible was returned into the oven for further drying. The drying, cooling and weighting were done at intervals of 4 hours until a constant weight was obtained. The moisture content was calculated as a percentage of the ratio of moisture loss to the weight of the samples analyzed. The expression represented below was used in the calculation:

Moisture (%) = $(w_1 – w_2/ w_1) \times 100$

where: W_1 = weight (g) of the sample before drying
W_2 = weight (g) of the sample after drying

2.2.2 Determination of ash content

Total ash content was determined as total inorganic matter by incineration of a sample at 600ºC [14]. Two (2 g) of the sample was weighed into a pre-weighed porcelain crucible and incinerated overnight in a muffle furnace at 600°C. The crucible was removed from the muffle furnace, cooled in desiccator and weighed. Ash content was calculated according to the following formula:

$$Ash\ (\%) = (\text{weight of ash})/(\text{Weight of sample}) \times 100\text{h}\ (\%) = \frac{\text{weight of ash}}{\text{Weight of sample}} \times 100$$

2.2.3 Dietary fibre

Using the method as described by Prosky *et al.* [15] , The samples were cooked at 100°C with heat stable α-amylase to give gelatinization, hydrolysis and depolymerization of starch; they were incubated at 60°C with protease (to solubilize and depolymerize proteins) and amyl glucosidase (to hydrolyze starch fragments to glucose); they were treated with four volumes of ethanol to precipitate soluble fibre and remove depolymerized protein and glucose (from starch). The residue was then filtered and washed with 78% ethanol, it was also washed with 95% ethanol, and finally acetone. After the washing, the residue was dried and weighed. One duplicate was analyzed for protein and the other was incubated at 525°C to determine ash. The Total Dietary Fibre was obtained by weighting the filtered and dried residue. The result was deducted from the weights of the protein and ash.

2.2.4 Determination of Crude Fat

Crude fat was estimated by employing solvent extraction using a Soxhlet extraction unit [14]. One gramme (1g) of the samples were weighed and placed in a thimble. Some 120 ml petroleum ether was poured into a previously dried and weighed round bottom flask. The Soxhlet extractor apparatus was set up with the flask and the condenser. The extraction apparatus was set up with the flask sitting on the spaces provided on the hot plate. The hot plate was plugged and set to gentle heating, the other evaporated and as it condensed, it dropped into the thimble where it extracted the other soluble constituents (fat constituent) into the flask. The colour deepened as time increases. The thimble was then removed and dried in the oven. The petroleum ether in the flask was evaporated. The flask was then dried in an air circulating desiccator. The round bottom flask and the lipid extract were then weighed. The flask and its content were dried again to obtain constant weight. Amount of lipid was obtained from the difference between the weight of the flask before extraction and after extraction. Crude fat was calculated using the formula:

$$\frac{(\text{weight offlask} + \text{oil}) - (\text{weight of flask}) \times 100}{\text{Weight of sample}}$$

2.2.5 Determination of crude protein

One gramme (1g) of the sample powder was weighed out into 50 ml Kjeldahl digestion flask. Some 20 ml concentrated H2SO4, 1 tablet of Kjeldahl catalyst and anti-bombing chips were added. The mixture was incinerated to gentle boiling on the digestion rack and then heated further for 3 hours. The digest was removed, cooled, quantitatively transferred to a 100 ml volumetric flask and made up to mark. Erlenmeyer flask containing 10 ml of the boric acid indicator solution was placed at the tip of the condenser extended below the surface of the solution. Ten millilitres (10 ml) of the sample digest was introduced into the sample tube and steam heated, 10 ml of 40% NaOH solution was added to the digest and the digest was steamed and distilled into the boric acid-indicator solution, it changed to green. A blank determination was

also carried out alongside that of the sample except that 1 g sample was replaced with 1ml distilled water. The crude protein content was calculated as follows:

Protein (%) = (A-B) × N × 1.4007 × 6.25

Where
A= volume (ml) of 0.2 N HCl used sample titration
B = volume (ml) of 0.2 N HCl used in blank titration
N= Normality of HCl
W = weight (g) of sample
14.007 = atomic weight of nitrogen
6.25 = the protein-nitrogen conversation factor

2.2.6 Calculation of available carbohydrate content

The available carbohydrate content of the samples was calculated by difference using the formula below [16].

Available carbohydrate = 100 – (crude protein + lipid + ash + moisture+ dietary fibre).

2.3 Determination of antinutrients

2.3 .1 Phytate determination

Phytate was determined by a simple and rapid colorimetric method by Latta and Eskin [17].

Five grams (5g) of the milled sample was weighed into a 250 ml conical flask, 100ml of 2.45HCL was added and extracted for 1 hour at room temperature $25^0C \pm 28^0c$ and centrifuged. Supernatant was decanted. 1ml of 2.4% extract supernatant was diluted to 25 ml with distilled water. Ten milliliters (10mls) of diluted sample was passed through the AG1-X8 chloride anion exchange column (0.5 g). Phytate was eluted with 0.7M NaCl. 3ml of 0.7M eluent fraction was pipetted into 15 ml conical test tubes, and mixed on a vortex mixer for 5 seconds, and centrifuged for 10 minutes. Absorbance of supernatant was read at 500 nm using water to zero the spectrophotometer. Series of sodium phytate dilutions were made from 5-40 µg phytate in distilled water. Three millimeters (3ml) of the solution was pipetted into 115ml. One millimeter (1ml) of Wade reagent was added within 30 minutes of elution. It was mixed on a vortex mixer for 5 seconds and centrifuged for 10 minutes. The absorbance of the supernatant was read at 500 nm using water to zero the spectrophotometer. Phytate content was estimated from the standard curve.

2.3.2 Determination of trypsin inhibitors

Trypsin inhibitor was determined using Kakade, Racis, Mcchee and Puski [18] method.

One hundred milligrams (0.01g) of sample was weighed; 20ml cold (4^oC) methanol was added. It was vortexed and centrifuged at 3,000rpm for 20minutes.

An aliquot of 0.01 to 5ml of supernatant was taken for assay. Some 0, 0.05, 0.1,0.2, 0.3, 0.4 and 0.5ml of estannic acid standard solution was pipetted into test tubes and made up to 5ml by adding 5, 4.95, 4.9, 4.8, 4.7, 4.6 and 4.5ml of distilled water (These correspond to concentrations of 0, 1, 2, 4, 6, 8 and10 ppm.) 0.3ml Folin-Denis reagent was added, 0.6ml of Na_2CO_3 solution was added. The solution stood for 25-30 mins. The absorbance of blue color was read at 760nm.

% Tannins was calculated from standard curve as follows:

$$\%\text{tannins} = \frac{(A-I)\, xVx100xD.\ F}{BxWx10^6}$$

A= Absorbance of sample.
I= Intercept
V= Total volume of extract

B= Slope of standard curve
W= Weight of sample

2.3.3 Oxalic acid determination

Oxalate was determined by the method of Oke [19]. One gram (1g) of the flour was extracted thrice by warming it at 40-50 degree centigrade with constant stirring with magnetic stirrer for 1 hour with 20ml of 0.3 N HCl The extract was diluted to 100ml with distilled water. 5ml of the extract was made alkaline with 1ml of 5 N NH4OH. This was made acidic with glacial acetic acid and phenolphthalein served as an indicator (2 drops). 1 ml of 5% calcium chloride was added and the mixture could stand for 3 hours, centrifuged using (IEC Centra GP8) at 1400 rpm for 15 minutes. The supernatant was discarded, and the precipitate was washed thrice with hot water, thorough mixing and centrifuging each time. 0.2ml of 3 N H2SO4 was pipette to each test tube.The precipitate was dissolved by warming in water at 70^{o}C for 30 mins. The content of each test tube was titrated with freshly prepared 0.01 N Potassium permanganate solutions. Titration was done at room temperature 29^{o}C until the colour of the solution become pink. The solution was allowed to stand until it became colourless. It was warmed at 70 degrees C and titrated until a pink color persisted for 30 seconds.
Calculations =oxalate content=W x 100/5

W= Mass of oxalate in 100ml

2.3.4 Determination of saponins

Saponins determination was carried out by Fenwick and Oakenfull [20] procedure. The sample was finely ground and dried at constant weight. 40g were weighed and placed in the Soxhlet reflux extractor with acetone for 24 hours. The solvent was changed for methanol and extraction was continued for another 24 hours. The methanolic extract was cooled and made to 250 ml with methanol. At this point there was a modification to the method, proposed by Miriam Monforte (CICY, Merida, Yucatan, Mexico). Instead of bringing the sample up to 250 ml as suggested in the original method it was concentrated. In this, methanolic extract was transferred into a rotary evaporator and concentrate until dry. The residue was concentrated again in a minimum of methanol and transferred to a reweighed vial. The vial was weighed with the dry sample and the weight of the residue was calculated. Fine drops of a standard solution of saponins were placed on the chromatography plates. The points of extract were placed so that each one is at the side of standard saponins drops. The plates were revealed and the drops with aspersion and a solution of sulphuric acid in methanol, it heated at 110°C for 30 min. The intensity of the saponins stains was measured with a densitometer and the peak areas were calculated on the plotter with a planimeter. The results were expressed as the relation (R) of the peak areas of the unknown sample in respect to those of the standard. R^2 was plotted against the volume of the drop of methanolized extract on the plate. The downslope of the line (was calculated by the least squares method), divided by the gradient of a line derived from a master curve, to give the concentration of saponins in the extract and thus the saponins content of the sample.

2.3.5 Determination of lectins by spectrometric method

Lectins was determined according to the method described by Brooks [21]. Two grammes (2g) of the sample were weighed into 40ml normal saline solution buffered at P^{H} 6.4 with 0.01m phosphate buffer solution. It could stand at room temperature for 30mins and centrifuged to obtain the extract. Half of a milliliter (0.5ml) of the extract was diluted in a test tube, 1 ml of heparinized rabbit blood was poured. The blank was prepared by adding 1ml of blood into a test tube and allowed to stand for 4h at room temperature. 1ml of normal saline was added to all the test tubes and it could stand for 10min. after which the absorbance was read at 620nm.

Lectin unit/g= (b-a) x F

Where b = absorbance of the blank

F= experimental factor given by

F= (1/w x f/va) D

Where

W= weight of sample
VF= total volume of extract
VA= volume of extract used in the assay
D= dilution factor

2.3.6 Raffinose and stachyose determination

Raffinose and stachyose (oligosaccharides) were determined by the method described by Tanaka, Thanakul, Lee, and Chichester [22].

Five grams each of both raw and processed flour were extracted with 50ml of 70% (v/v) aqueous ethanol and kept on an orbital shaker at 130 rpm for 13h. Extracts were further washed with 25ml of 70% (v/v) ethanol. The filtrates obtained were then concentrated on a water bath. The concentrated sugar syrup was dissolved in 5ml of distilled water. Separation of oligosaccharides was done by thin layer chromatography (TLC). A 100g silica gel was dissolved in distilled water and stirred well until the slurry was homogeneous. The TLC plates were washed, dried and cleaned with chloroform to remove any grease from the plates. TLC plates were then coated with the slurry and air-dried. Spotting of the sugar samples was done by using capillary tubes. Each sample was spotted twice separately and dried using electronic hand drier. The plate was developed by using a solvent system of n-propanol, ethyl acetate and distilled water (6:1:3), and dried. The separated sugars' colours were developed with iodine crystals. The separated spots were compared with the standard sugar spots. The separated sugars that appeared were stachyose, raffinose and sucrose. The stachyose and raffinose spots were scrapped, eluted in 2ml of distilled water, kept overnight and filtered through Whatman No.1 filter paper. The filtrates were then subjected to quantitative estimation. The eluted individual oligosaccharide was estimated. One ml of the eluted and filtered sugar solution was treated with one ml of concentrated HCl. The tubes were boiled in water bath for exactly 6 min. After cooling, absorbances of the oligosaccharide contents were read using spectrophotometer 259 at 432nm. The absorbance values were used to calculate the concentration and mass of the oligosaccharides. Average values of duplicate estimations were calculated, and the oligosaccharide contents expressed on a dry weight basis.

2.4 Gruel production

Gruels were prepared from the processed AYB flour (whole and dehulled)

Recipe for gruel

	Quantity (g)
Ingredients	
AYB flour	39
Corn	14
Water	329
Canderel zero calorie sweetener	0.7
Yield	**350**

Method of preparation

i. Seventy grams (70g) of tap water was used to reconstitute the flour
ii. Water (259mls) was brought to the boil.
iii. The boiled water was gradually added to the reconstituted flour while stirring continuously to avoid the formation of lumps.
iv. The mixture was returned to the pot, placed on fire and allowed to simmer gently
v. The mixture was simmered for 3 minutes stirring continuously till cooked.
vi. Artificial sweetener was added and stirred
vii. It was served hot

2.5 Sensory evaluation

In combination with an affective test, multiple comparison tests were done, using trained panelist at the preliminary stage to screen treatments and select the best concentration of citric acid, fermentation time and/or ratio of flour to use for final product development. After the products were developed, Consumer acceptability of the products were assessed using the affective test based on individual acceptability or preferences. It also involves the rating/acceptance of the sample using a 9- point hedonic scale to determine the degree of acceptability of the new products [23,24]

2.6 Statistical Analysis

An IBM Statistical Package for Social Sciences (SPSS) database version 20.0 computer was used to analyze the data. The analysis of variance (ANOVA) was used where descriptive statistics like means was used to analyze the continuous variables and standard deviations were calculated to show the statistical variability. Post Hoc was performed and means were compared using the least significant ($P<0.05$) difference (LSD).

3. Results and discussion

Effect of varying fermentation time on the proximate composition of whole and dehulled AYB flours fermented in 0.05% citric acid solution (% dry weight basis).

Table 1 showed the effect of varying fermentation time on the proximate composition of treated (0.5% citric acid) whole and dehulled AYB flours. The moisture content (10.2-10.5) of the raw and 24hr fermented whole and dehulled AYB were statistically ($P>0.05$) similar, but differ significantly ($P<0.05$) from those fermented for 48 and 72 hours. The moisture contents of the processed flours were all within the safe levels for storage [25]. The moisture content of food is a good indicator for ensuring its keeping quality. Dehulled AYB fermented in citric acid solution for 24hrs had the highest protein (30.5%). The 24h and 72h whole and 72hr dehulled AYB fermented in citric acid had similar protein values (26.9%, 26.3% and 27.1% respectively). Raw untreated AYB had the least protein value (20%). The significant increase observed in the protein content of the flours especially dehulled AYB fermented for 24hrs in 0.5% citric acid medium is not surprising as studies have indicated an increase in the protein contents of fermented foods [26, 27]. The result can also be attributed to the citric acid used; fermentation in citric acid medium have been shown to significantly increase the protein contents of foods, this increase can be attributed to the low pH provided by the citric acid thereby facilitating protein solubilization, resulting in higher protein yields [28,29]. The length of fermentation and dehulling might have contributed to the protein increase because the fermentation time (24hrs) might not have permitted leaching of the proteins in the fermentation medium and also because proteins are more concentrated in the cotyledon, therefore dehulling reduces the bulking effect and makes the proteins concentration of the dehulled samples higher [30]. The fat contents significantly ($P<0.05$) decreased with the treatment. Raw untreated AYB had 1.6% fat while whole AYB fermented for 72hr had 1% fat. The decreases in the fat content of the AYB flour samples are in line with several studies that reported decreases in fat content in fermented products [31], the low-fat content of the processed flours further enhance the keeping quality of the flour as rancidity will be reduced [32]. The ash contents also decreased with processing even though they were slight variations. They ranged from 2.3% in dehulled AYB fermented for 72hrs to 2.7% in raw AYB, Ash content indicates the level of mineral element [33]. The dietary fibre values varied significantly ($P<0.05$) (14.3 – 17.5%). There was a decrease with processing from17.5% in raw AYB to 14.3% in dehulled AYB fermented for 72hrs. The reduction in the dietary fibre of all the AYB flours especially the dehulled sample was expected as more dietary fibre will be expected in the whole AYB sample and studies have reported

reduction in the fibre contents of fermented legumes [34]. All the fermented samples had significant reduction in their carbohydrate contents; this was expected with the increase in protein and in some cases fat.

TABLE 1

Effect of varying fermentation time on the proximate composition of treated (0.5% citric acid) whole and dehulled AYB flours (%).

	Moisture	Protein	Fat	Ash	Dietary Fibre	Available CHO
Raw AYB	10.2 ± 0.02[a]	20.0 ±1.4[d]	1.6± 0.10[a]	2.7± 0.02[a]	17.5 ± 0.35[a]	48.0 ± 0.15[a]
24h fermented whole AYB	10.5 ± 0.00[a]	26.9[b] ± 1.19[b]	1.1[b] ± 0.02[b]	2.6 ± 0.05[b]	17.2±0.00[a]	41.7 ± 0.02[d]
24h fermented dehulled AYB	10.2 ± 0.00[a]	30.5 ± 0.57[a]	1.2± 0.02[a]	2.5 ± 0.02[a]	15.4±0.04[c]	40.2 ± 0.02[a]
48h fermented whole AYB	9.3± 0.00[b]	21.2 ± 0.10[d]	1.1± 0.12[b]	2.5 ± 0.00[c]	17.0±0.12[a]	48.9 ± 0.02[a]
48h fermented dehulled AYB	8.4 ± 0.01[c]	24.5 ± 0.24[c]	1.2± 0.05[b]	2.4 ± 0.02[d]	15.2±0.08[c]	48.3 ± 0.01[a]
72h fermented whole AYB	9.2 ± 0.20[b]	26.3 ± 0.03[b]	1.0± 0.01[c]	2.4 ± 0.05[d]	16.4±0.12[b]	44.7 ± 0.01[c]
72h fermented dehulled AYB	9.1 ±0.00[b]	27.1 ± 0.02[b]	1.1± 0.10[b]	2.3 ± 0.02[e]	14.3 ±0.00[d]	46.1 ± 0.02[b]

***Means of 3 replicates. Values are expressed as mean ± S.D. [a-h] values with different superscripts on the same column are significantly different (p<0.05).**

Effect of treatment and fermentation time on the anti-nutrient content of AYB flour samples

Table 2 presents the effect of treatments and fermentation on the anti-nutrient and toxic substance composition of AYB flour samples. Results are reported at p<0.05 level of significance.

Citric acid fermented samples had significantly (P<0.05) reduced trypsin inhibitors (TI) when compared to the raw AYB sample. The reduction was 1.85 IU/mg in the raw AYB to 0.05 IU/mg in whole AYB fermented for 48hrs in citric acid solution. The trypsin inhibitors in the flours were all reduced after treatment and fermentation. The level of reduction might be as a result of the treatment with citric acid as an acidic medium are known to lead to hydrolysis of many anti-nutrients and toxic substances leading to improved nutrient utilization [35]. Trypsin inhibitors levels of the treated and fermented AYB were within the safe level of less than 0.54 IU/mg [36] and can, therefore, be regarded as safe for consumption.

The raw AYB sample had significantly (P<0.05) higher (5.1mg/g) phytate content compared to the treated samples. All AYB samples fermented in citric acid solution had significantly reduced phytate level and they ranged from 2.2mg/g in the whole AYB fermented for 24hrs citric acid solution to 1.1mg/g in dehulled AYB fermented for 48hrs in citric acid solution. Phytate was reduced to safe levels in all the

fermented samples. Studies have shown that reduction of phytate levels in food are reduced to about 4.9mg/g, brings about five folds increase in the bioavailability of iron [37]. The phytate levels of the products were reduced to about three times this cited low level. Also, the phytate-zinc molar ratios observed from this study were very low indicating a high bioavailability of zinc. Therefore, the phytate level of the flours and their products might be incapable of chelating calcium or limiting the bioavailability of iron or zinc [38,39]. Citric acid treatment of AYB must have contributed to the reduction of phytic acid as it lowered the pH of the fermented flour; and studies have shown that phytic acid reduction is aided by low pH [40].

The oxalate level of the samples followed similar trends with other antinutrients as the raw samples had significant ($P<0.05$) the highest oxalate level of 0.21mg/g, while the samples fermented for 72hrs had significantly ($P<0.05$) the lowest oxalate level of 0.01mg/g. The levels of oxalates in both fermented AYB and corn are low [41], therefore can be safe because the lethal dose of oxalate is levels above 100 mg/100g [42]. High oxalates in food cause irritation in the mouth or interfere with iron or calcium absorption [43]. The levels of oxalate observed in this study are not likely to either interfere with iron or calcium absorption or lead to the formation of stones in the urinary tract.

Saponins were significantly reduced in all the processed samples. The reduction was from 0.3mg/100g in the raw sample to trace (0.00mg/100g) in 48hrs and 72hrs fermented samples. The saponin levels observed in all the samples were low and within safe levels. A study on the lethal dose of saponin was observed to be 200 mg/kg [44]. All the studied flours could be regarded as safe for consumption and the products might not exact negative effects like hemorrhage and erosion of the mucosa of the small intestine or necrosis of liver cells and renal tubules was attributable to the consumption of saponins [45].

Tannins were also significantly ($P<0.05$) reduced in all the processed samples hence the reduction was from 0.9 mg/g in raw AYB to 0.01mg/g in dehulled AYB fermented for 72hrs in 0.5% citric acid. The concentration of raffinose was significantly ($P<0.05$) reduced with processing. It was higher (2.18%) in the raw sample than the samples fermented in citric acid solution, but significantly ($P<0.05$) lowest (1.38%) in dehulled AYB fermented for 72hrs. The significant reduction in the tannin contents of the processed samples might have contributed to the acceptability of the products as products with high tannin levels are known to have bitter taste thus reducing consumer choice for such foods [45, 46]. Tannin levels in the range of 0.02mg/g - 0.05mg/g are regarded as low suggesting that these products might not form complex with protein, starch, cellulose or minerals because of the significant reduction of the tannin content [47].

Fermentation in citric acid medium had significant ($P<0.05$) impact by reducing the stachyose contents of the treated samples in comparison with the raw sample. The raw AYB (3.16%) had significantly ($P<0.05$) higher stachyose than all other samples. There was no significant difference in the level of stachyose in 24hrs and 48hrs fermented samples. Dehulled AYB fermented for 72hrs had the least level (0.01%) of stachyose than all other samples. The significant reduction in stachyose and raffinose in all the processed samples, when compared with the raw AYB is in line with a study by Chen [48] that reported a similar reduction in the oligosaccharide content of fermented soybean. This reduction is an indication that the diets might have less toxicological and nutritional problems like diarrhea, gas production with belching, flatulence, abdominal bloating and pain [49]. From the study, fermentation time influenced stachyose and raffinose reduction, that is, the longer the fermentation time, the more reduced the oligosaccharide content. The significant reduction of haemagglutinin in all the samples could be attributed to fermentation. Haemagglutinin was significantly reduced from 32 Hu/100g in the raw sample to 4.56 Hu/100g in dehulled AYB fermented in 0.5% citric for 72hr. Studies have found haemagglutinin to be most unstable to traditional processing like fermentation, soaking, cooking, germination among others and that a processed legume will hardly exert any toxic effects associated with foods that contain hemagglutinin [50], other studies have stated that there is no evidence that hemagglutinin in food will have any toxic effect after cooking.

TABLE 2

The effect of varying fermentation time on the antinutrient and toxic substance composition of treated (0.5% citric acid) whole and dehulled AYB flours (Dry weight basis)

	Raw AYB	24hrs WAYB	24hrs DAYB	48hrs WAYB	48hrs DAYB	72hrs WAYB	72hrs DAYB
Trypsin inhibitors (IU/mg)	1.85±0.04 [a]	0.06 ± 0.00 [b]	0.3±0.01 [e]	0.05±0.00 [e]	0.15±0.01 [d]	0.06 ± 0.01 [e]	0.26 ± 0.03 [c]
Phytate (mg/g)	5.1 ± 0.16 [a]	2.2 ± 0.2 [b]	1.6 ± 0.16 [c]	1.6 ± 0.04 [c]	1.1 ± 0.06 [d]	1.6 ± 0.05 [b]	1.5 ± 0.08 [b]
Oxalate (mg/g)	0.21 ± 0.01 [b]	0.08 ± 0.02 [b]	0.06 ± 0.02 [cd]	0.07 ± 0.01 [d]	0.05 ± 0.0 [d]	0.02 ± 0.01 [e]	0.01 ± 0.00 [e]
Saponin (mg/100 g)	0.3± 0.02 [a]	0.01± 0.02 [b]	0.01± 0.01 [b]	0.00± 0.00 [c]	0.00± 0.02 [c]	0.00± 0.02 [c]	0.00± 0.02 [c]
Tannins (mg/g)	0.9±0.3 [a]	0.07±0.01 [d]	0.05±0.00 [d]	0.04±0.0 [c]	0.02±0.02 [cd]	0.03±0.01 [b]	0.01±0.02 [bc]
Raffinose (%)	2.18± 0.02 [a]	1.76±0.02 [b]	1.56±0.01 [c]	1.76±0.00 [a]	1.56±0.02 [c]	1.58±0.00 [c]	1.38±0.00 [c]
Stachyose	3.16 ± 0.03 [a]	0.05± 0.01 [b]	0.03±0.02 [bc]	0.03±0.02 [bc]	0.02± 0.04 [bc]	0.02± 0.02 [bc]	0.01± 0.04 [c]
Lectins (Hu/100 g)	32.46±3.00 [a]	5.52±0.02 [b]	4.70±0.05 [c]	5.34±0.02 [b]	5.31±0.01 [b]	5.51±0.01 [b]	4.56±0.01 [c]

DAYB- dehulled AYB, WAYB- whole AYB
***Means of 3 replicates. Values are expressed as mean ± S.D. [a-e] values with different superscripts on the same row are significantly different (p<0.05).**

Table 3 presents the effect of different concentration of citric acid (0.25%, 0.5% & 1%) and varying fermentation time on the sensory characteristics of whole and dehulled AYB gruel. Whole and dehulled AYB fermented for 24hrs with 0.5% citric acid had significantly (P< 0.05) higher scores for aroma (7.30, 7.35, respectively) compared to those fermented for 48hrs and 72hrs; with samples fermented at 72hrs having the least scores. This result can be attributed to the fermentation time and concentrations of citric acid. As the length of fermentation increases, the score for aroma decreases. This is evident in the least score for aroma observed in the samples that were fermented for 72hrs. on the other hand, dehulled AYB fermented for 24h using 1% citric acid had significantly (P<0.05) the highest score for colour (7.99) while dehulled AYB fermented for 24hrs in 0.25% citric acid had the least score (5.26) for colour. This result is not surprising as International Food Information Council (IFIC) and Food and Drug Administration [51] noted that citric acid can be used to improve colour, more so, the improved colour in the sample with the highest concentration of citric acid (1%) could be linked to the concentration. In terms of taste, AYB fermented for 24hrs with 0.5% citric acid had a significantly higher score (7.79, 7.81) than all other treated samples. It was observed that higher concentration of this acid lead to sour taste in the product limiting their acceptability. No significant difference (p>0.05) was observed for texture. Gruel made from whole and dehulled AYB fermented for 24hrs in 0.5% citric acid was the most acceptable of all the samples, having a significantly higher score of 7.32 & 7.22 respectively, followed by AYB fermented for 24hrs in 1% citric acid solution.

Table 3: The effect of different concentration of citric acid (0.25%, 0.5% & 1%) and varying fermentation time on the sensory characteristics of whole and dehulled AYB gruel

Samples	Aroma	Colour	Taste	Texture	General acceptability
Whole AYB fermented for 24hr (0.25% citric acid)	5.83±1.40 [c]	6.59± 1.01 [ab]	5.61±1.14 [c]	7.00±1.12 [a]	5.33±1.26 [c]

Dehulled AYB fermented for 24hrs (0.25% citric acid)	5.85± 1.11[c]	7.59±1.06[ab]	5.59±1.15[c]	7.04±1.14[a]	5.32±1.23[c]
Whole AYB fermented for 24hrs (0.5% citric acid)	7.38 ±1.21[a]	6.66±1.04[ab]	7.79±1.28[a]	7.16±1.16[a]	7.32±1.10[a]
Dehulled AYB fermented for 24hrs (0.5% citric acid)	7.39±1.12[a]	7.60±1.09[ab]	7.81±1.14[a]	7.12±1.18[a]	7.22±1.17[a]
Whole AYB fermented for 24hrs (1% citric acid)	7.40±1.14[a]	6.87±1.42[ab]	6.81±1.49[b]	6.78±1.09[a]	6.56±1.22[b]
Dehulled AYB fermented for 24hrs (1% citric acid)	7.40±1.28[a]	7.99±1.13[a]	6.78±1.01[b]	7.01±1.14[a]	6.54±1.06[b]
Whole AYB fermented for 48hrs (0.25% citric acid)	5.55±1.14[d]	5.95±1.70[de]	5.03±1.28[c]	7.19±1.13[a]	5.25±1.11[c]
Dehulled AYB fermented for 48hrs (0.25% citric acid)	5.65±1.19[d]	6.01±1.14[cd]	5.00±1.07[c]	7.23±1.42[a]	5.23±1.31[c]
Whole AYB fermented for 48hrs (0.5% citric acid)	6.47±1.30[b]	6.05±1.35[cd]	6.35±1.56[b]	7.01±1.28[a]	6.14±1.12[b]
Dehulled AYB fermented for 48hrs (0.5% citric acid)	6.50±1.02[b]	6.10±1.70[cd]	6.30±1.42[b]	7.11±1.45[a]	6.09±1.02[b]
Whole AYB fermented for 48hrs (1% citric acid)	6.62±1.19[b]	6.01±1.14[cd]	6.33±1.03[b]	7.10±1.07[a]	6.10±1.09[b]
Dehulled AYB fermented for 48hrs (1% citric acid)	6.68±1.10[b]	6.12±1.28[c]	6.22±1.57[b]	7.13±1.42[a]	6.06±1.32[b]
Whole AYB fermented for 72hrs (0.25% citric acid)	4.77±1.10[e]	5.40±1.84[f]	4.40±1.85[d]	7.02±1.28[a]	4.50±1.10[d]
Dehulled AYB fermented for 72hrs (0.25% citric acid)	4.82±1.10[e]	5.26±1.10[f]	4.32±1.28[d]	7.14±1.28[a]	4.46±1.21[d]
Whole AYB fermented for 72hrs (0.5% citric acid)	4.80±1.28[e]	5.40±1.28[f]	5.40±1.56[c]	6.98±1.58[a]	4.94±1.15[d]
Dehulled AYB fermented for 72hrs (0.5% citric acid)	4.85±1.45[e]	5.44±1.23[f]	5.39±1.42[c]	7.00±1.15[a]	4.91±1.06[d]
Whole AYB fermented for 72hrs (1% citric acid)	4.90±1.63[e]	5.80±1.15[d]	5.70±0.97[c]	6.69±1.20[a]	5.05±1.10[bd]
Dehulled AYB fermented for 72hrs (1% citric acid)	4.95±1.33[e]	5.83±1.12[ef]	5.67±0.90[c]	7.01±1.15[a]	5.00±1.14[bd]

***mean of 10 panelist response on a 9-point hedonic scale. [a-d] values with different superscripts on the same column are significantly differen**t ($p<0.05$). Organoleptic Scores/rating 1. Dislike extremely, 2. Dislike very much, 3. Dislike moderately, 4. Dislike slightly, 5. Neither like nor dislike, 6. Like slightly, 7. Like moderately, 8.Like very much, 9. Like extremely

Table 4 presents the effect of varying fermentation time on the sensory characteristics of gruels from whole and dehulled AYB fermented in citric acid solution and tap water.

When compared to other samples, gruels from whole and dehulled AYB fermented for 24hrs in 0.5% citric acid solution had significantly ($P< 0.05$) higher scores for aroma (7.70, 7.65), colour (6.99, 7.10), taste (7,78, 7.00) and general acceptability (7.54, 7.23) respectively than all the samples that were not treated with citric acid but were fermented in tap water. No significant difference ($p>0.05$) was observed for texture. Gruels of whole and dehulled AYB fermented for 72hrs in tap water had the least value for aroma (4.44, 4.55), colour (4.83, 4.87) and general acceptability status (4.00, 3.99) respectively. The high acceptability status of the gruel made from AYB that was fermented in 0.5% citric acid solution for 24hrs could be as a result of the

treatment with citric acid and the concentration used, as citric acid is known to improve colour, aroma and enhance the taste of foods [51, 52, 53].

Table 4: Effect of varying fermentation time on the sensory characteristics of treated (0.5% citric acid) and untreated whole and dehulled AYB gruels

	Aroma	Colour	Taste	Texture	General acceptability
Whole AYB fermented for 24hrs	5.22±1.20 ^{c}	6.65±1.02 ^{ab}	5.51±1.33 ^{c}	7.02±0.04 ^{a}	5.12±1.12 ^{c}
Dehuled AYB fermented for 24hrs	5.35±1.24 ^{c}	6.66±1.33 ^{ab}	5.49±1.45 ^{c}	7.11±0.50 ^{a}	5.22±1.15 ^{c}
Whole AYB fermented for 24hrs (0.5% citric acid)	7.70±1.23 ^{a}	6.99±0.98 ^{a}	7.78±1.55 ^{a}	7.11±0.19 ^{a}	7.54±0.32 ^{a}
Dehulled AYB fermented for 24hrs (0.5% citric acid)	7.65±1.12 ^{a}	7.10±1.05 ^{a}	7.00±1.01 ^{a}	7.13±0.18 ^{a}	7.23±0.15 ^{a}
Whole AYB fermented for 48hrs	5.50±1.30 ^{c}	6.01±1.02 ^{cd}	5.30±1.15 ^{c}	7.01±0.19 ^{a}	5.09±0.91 ^{c}
Dehulled AYB fermented for 48hrs	5.48±1.40 ^{c}	6.02±1.11 ^{cd}	5.22±1.12 ^{c}	7.13±0.64 ^{a}	4.86±0.82 ^{c}
Whole AYB fermented for 48hrs (0.5% citric acid)	6.85±1.19 ^{b}	6.44±1.10 ^{bc}	6.39±1.11 ^{b}	7.00±0.61 ^{a}	6.51±0.81 ^{b}
Dehulled AYB fermented for 48hrs (0.5% citric acid)	6.82±0.01 ^{b}	6.56±1.12 ^{bc}	6.32±1.21 ^{b}	7.14±0.12 ^{a}	6.46±0.71 ^{b}
Whole AYB fermented for 72hrs	4.44±1.18 ^{d}	4.83±1.21 ^{e}	4.67±112 ^{c}	7.01±0.04 ^{a}	4.00±0.62 ^{d}
Dehulled AYB fermented for 72hrs	4.55±1.11 ^{d}	4.87±1.21 ^{e}	4.33±1.14 ^{d}	7.04±0.02 ^{a}	3.99±0.94 ^{d}
Whole AYB fermented for 72hrs (0.5% citric acid)	5. 23±1.17 ^{d}	5.68±1.30 ^{d}	5.04±1.02 ^{c}	7.03±0.12 ^{a}	4.99±0.57 ^{c}
Dehulled AYB fermented for 72hrs (0.5% citric acid)	5.32±1.20 ^{c}	5.70±1.36 ^{d}	5.03±1.12 ^{c}	7.23±0.15 ^{a}	4.66±0.85 ^{c}

***mean of 10 panelist response on a 9-point hedonic scale. $^{a-d}$values with different superscripts on the same column are significantly different (p<0.05).** Organoleptic Scores/rating 1. Dislike extremely, 2. Dislike very much, 3. Dislike moderately, 4. Dislike slightly, 5. Neither like nor dislike, 6. Like slightly, 7. Like moderately, 8.Like very much, 9. Like extremely

4. Conclusion

The findings from this study have shown that fermenting AYB seeds in 0.5% citric acid for 24hrs will produce wholesome flours free from beany flavour and bitter taste. Additionally, these flours will enhance the food use of AYB. AYB being an underutilized crop which fears extinction might be revived and used in variable ways. The gruels made from flours fermented in 0.5% citric acid for 24hrs is shown to have a considerable acceptability compared to flours produced from AYB seeds that were fermented in 0.25% and 1% citric acid as well as tap water.

Although, all the fermented flours had increased protein contents and reduced fat, dietary fibre and antinutrients, the AYB fermented in 0.5% citric acid for 24hrs is recommended because of its higher protein, dietary fibre and sensory quality.

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