



Effect of Citric Acid Treatment and Fermentation on the Chemical Composition of African Yam Bean (*Sphenostylis stenocarpa*) and Sensory Evaluation of Its Gruel

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Authors' contributions

All authors collaborated to get this work done. Author LCA conceived the work and designed the initial protocol, did the literature review, laboratory works and statistical analysis and wrote the draft of the manuscript. Author HNEO modified the protocol and supervised every step of the study and made sure the protocol was well executed. Author EDO read through the whole manuscript and made technical and grammatical input. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Legumes, (especially indigenous/ underutilized legumes), have been found to make substantial contributions to nutrient intakes of the populace especially in low resources environments. The African yam bean (AYB) is one of such indigenous legumes with utilization constraints such as hard-to-cook phenomenon, beany flavour, bitter taste and some anti-nutritional factors that limit its utilization. The aims of this study were to determine effect of citric acid treatment and fermentation on the chemical composition of African yam bean (*Sphenostylisstenocarpa*) and sensory evaluation of its gruel.

Methodology: Cream coloured AYB seeds were purchased in Enugu, Enugu State Nigeria. Sorted AYB seeds were washed and fermented in citric acid medium (0.25%, 0.5% and 1%) for 24 h 48 h

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and 72 h at room temperature (28°C) in a seed water ratio of 1:4 (w/v). The control seeds were fermented without citric acid for 24 h, 48 h and 72 h. After fermentation, each batch of the fermented seeds was divided into two. The first half was dehulled and the other half was left as whole. The fermented seeds were separately dried and milled into flour for further use. Standard methods were used for proximate, mineral and anti-nutrient analyses. Gruels were produced from all the flours and subjected to sensory evaluations using a nine-point hedonic scale. Data obtained were analyzed using a Statistical Package for Social Sciences (SPSS) 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 $P < .05$.

Results: Chemical compositions of AYB flour 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 raw and dehulled AYB seeds fermented for 24 h with 0.5% citric acid had higher scores for aroma (7.30 & 7.35, respectively) and general acceptability (7.32 & 7.22, respectively). Dehulled AYB fermented for 24 h in 1% citric acid had the highest score (7.99) for colour. Based on the sensory evaluation results, gruels made from the AYB seeds fermented in 0.5% citric acid compared with the gruels made from AYB seeds that were fermented in tap water. The results showed that gruels made from the AYB seeds that were fermented in 0.5% citric acid for 24 h had higher score for aroma (7.70), colour (7.10) and overall acceptability (7.52). Utilization of AYB in food formulation could be improved by fermenting in 0.5% citric acid solution for 24 h.

Keywords: Citric acid; fermentation; proximate; anti-nutrient; sensory evaluation; underutilized legumes; 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 food materials has been proposed [4]. This is because awareness creation on utilization of indigenous foods could help to improve nutritional status of the consumers and add variety to their diets [5]. Eating nutritious food is essential to achieve good health. Africa Yam bean (AYB) (*Sphenostylisstenocarpa*) has been cited as one of the underutilized 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 on the liver [10]. Nutritionally, AYB has higher amino acid profile than most legumes [11]. Its protein concentrate is reportedly used for the fortification of starchy foods; despite all these attributes, its utilization is limited by its beany flavour, bitter taste, long cooking time of about 6 h and flatulence inducing oligosaccharides [12,13].

This study has adopted the use of different concentrations of citric acid to produce a whole some flour free of beany flavour and bitter taste. The use of these flours to produce gruels has minimized the problem of long cooking time of about 6 h.

2. MATERIALS AND METHODS

2.1 Preparation of African Yam Bean Flour

Cream coloured AYB seeds were purchased from Enugu in Enugu State, Nigeria. The seeds were sorted to remove impurities and weighed. From the sorted AYB seeds, 20 kg was weighed, washed and fermented in citric acid medium

(0.25%, 0.5% and 1%) for 24 h, 48 h and 72 h at room temperature (28°C) in a seed to citric acid solution ratio 1:4 (w/v). At the end of fermentation, AYB seeds were washed, each batch was divided into two and one-half of the fermented portions was dehulled, while the remaining were left as whole. They were all placed in separate compartments in a food dehydrator (DH6 5NG, UK), set at 70°C for 4 h to dry up excess water and prevent further fermentation. The temperature was reduced to 40°C for 44 hrs. The dried seeds were milled into fine flours using (70 mm mesh screen) 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 2010 [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 [16].

2.2.1 Determination of moisture content

Two grams (2 g) of the sample was weighed into a previously weighed crucible. The crucible plus the sample was taken and transferred into the oven set at 100°C 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:

$$\text{Moisture (\%)} = (w_1 - w_2 / w_1) \times 100$$

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

600°C [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:

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

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 (1 g) 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:

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

2.2.5 Determination of crude protein

One gramme (1 g) 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 1 ml distilled water. The crude protein content was calculated as follows:

$$\text{Protein (\%)} = (A-B) \times N \times 1.4007 \times 6.25$$

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 content of sample was determined followed a simple and rapid colorimetric method as described by Latta and Eskin [17]. Five grams of the milled sample was weighed into 250 ml conical flask, 100 ml of 2.45 M HCl was added, extracted for 1 h at room temperature (25°C±2°C) and centrifuged. Supernatant was decanted, 1ml of 2.4% supernatant extract was diluted to 25 ml with distilled water. Ten milliliters 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 sec, and centrifuged for 10 min. Series of sodium phytate dilutions were made from 5-40 µg phytate in distilled water. Three millimeters of the solution was pipetted into 100 ml conical flask. One millimeter of Wade reagent was added within 30 min of elution. It was mixed on a vortex mixer for 5 sec and centrifuged for 10 min. The absorbance was read at 500 nm using water to standardize 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 of sample was weighed; 20 ml cold (4°C) methanol was added. It was vortexed and centrifuged at 3,000 rpm for 20min. An aliquot of 0.01 to 5 ml 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 5 ml by adding 5, 4.95, 4.9, 4.8, 4.7, 4.6 and 4.5 ml of distilled water (These correspond to concentrations of 0, 1, 2, 4, 6, 8 and 10 ppm) From Folin-Denis reagent, 0.3ml was added, 0.6 ml of Na₂CO₃ solution was also added. The solution stood for 25-30 mins. The absorbance of blue color was read at 760 nm.

% Tannins was calculated from standard curve as follows:

$$\% \text{Tannins} = \frac{(A - I) \times V \times 100 \times D.F}{B \times W \times 10^6}$$

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

B = Slope of standard curve
 W = Weight of sample
 D.F = Dilution factor

2.3.3 Oxalic acid determination

Oxalate content was determined following the method of Oke [19]. One gram of the flour was extracted thrice by warming it at 40-50°C in a water bath with constant stirring using magnetic stirrer for 1 h with 20 ml of 0.3 N HCl. The extract was diluted to 100 ml with distilled water. Five milliliters of the extract was made alkaline with 1 ml of 5 N NH₄OH. This was made acidic with glacial acetic acid and 2 drops of phenolphthalein was added as an indicator. 1 ml of 5% calcium chloride was added and the mixture was rested for 3 h, centrifuged using (IEC Centra GP8) at 1400 rpm for 15 min. The supernatant was discarded, the precipitate was washed thrice with hot water, mixed thoroughly, and centrifuged each time. Thereafter, 0.2 ml of 3 N H₂SO₄ was pipetted to each test tube and precipitate was dissolved by warming in water bath at 70°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°C) until the colour of the solution become pink. The solution stood until it became colourless. It was warmed at 70°C and titrated until a pink color persisted for 30 secs.

$$\text{Calculations} = \text{oxalate content} = W \times 100/5$$

W= Mass of oxalate in 100ml

2.3.4 Determination of saponins

Saponins determination was carried out by following Fenwick and Oakenfull [20] procedure with modification. The sample was finely ground and dried at constant weight. From dried sample, 40 g was weighed and placed in Soxhlet reflux extractor with acetone for 24 h. The solvent was changed to methanol and extraction was continued for another 24 h. Why? The methanolic extract was cooled and made up to 250 ml with methanol. This was modified by concentrating the sample instead of bringing the sample up to 250 ml as suggested in the original method. The methanolic extract was transferred into a rotary evaporator and concentrated until dry. The residue was concentrated again 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 that of the standard. R² 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 standard 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 of the sample were weighed into 40 ml normal saline solution buffered at pH 6.4 with 0.01 M phosphate buffer solution. It was rested at room temperature for 30 mins and centrifuged to obtain the extract. Half of a milliliter of the extract was diluted in a test tube, 1 ml of heparinized rabbit blood was poured. The blank was prepared by adding 1 ml of the blood into a test tube and allowed to stand for 4 h at room temperature. From normal saline, 1 ml was added to all the test tubes and rested for 10 min, after which the absorbance was read at 620 nm.

$$\text{Lectin unit/g} = (b-a) \times F$$

where,

b = absorbance of the blank
 F = experimental factor given by
 $F = (1/w \times f/va) D$

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

Oligosaccharides (raffinose and stachyose) contents 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 50 ml of 70% (v/v) aqueous ethanol and kept on an orbital shaker at 130 rpm for 13 h. Extracts were further washed with 25 ml of 70% (v/v) ethanol. The filtrates obtained were then concentrated on a water bath. The concentrated sugar syrup was dissolved in 5 ml of distilled water. Separation of oligosaccharides was done by Thin Layer Chromatography (TLC). A 100 g 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 measured and the eluted individual oligosaccharide was estimated. One milliliter of the eluted and filtered sugar solution were treated with 1 ml of concentrated HCl. The tubes were boiled in water bath for exactly 6 min at what temperature. After cooling, the absorbances of the oligosaccharide contents were read using spectrophotometer (A. Analyst 300, Perkin Elmer-U.S.A) 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 Preparation of Gruel

Method of preparation:

- i. Seventy grams (70 g) of tap water was used to reconstitute the flour
- ii. Water (259 mls) 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

Chart 1. Gruels were prepared from the processed AYB flour (whole and dehulled)

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

2.5 Sensory Evaluation

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 ratio of flour to use for final product development. After the products were developed, consumer acceptability of the products was assessed using the semi-trained panelist to assess the samples on 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) 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

3.1 Effect of Varying Fermentation Time on the Proximate Composition of Whole and Dehulled AYB Flours Fermented in 0.5% 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 24 h fermented whole and dehulled

AYB were not significantly ($P>0.05$) different, but differ significantly ($P<0.05$) from those fermented for 48 and 72 h. 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 24 h had the highest protein (30.5%). The 24 h and 72 h whole and 72 h dehulled AYB fermented in citric acid had similar protein values (26.9%, 26.3% and 27.1% respectively). Raw AYB had the least protein value (20%). The significant increase observed in the protein content of the flours especially dehulled AYB fermented for 24 h 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 (24 h) 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 AYB had 1.6% fat, while whole AYB fermented for 72h 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 there were slight variations. They ranged from 2.3% in dehulled AYB fermented for 72h to 2.7% in raw AYB, Ash content indicates the level of mineral element [33]. The dietary fibre values varied significantly ($P<0.05$) from 14.3 to 17.5%). There was a decrease in dietary fibre from 17.5% in raw AYB to 14.3% in dehulled AYB fermented for 72h. 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.

3.2 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 ranged from 1.85 IU/mg in the raw AYB to 0.05 IU/mg in whole AYB fermented for 48h 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 cause 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 thus, be regarded as safe for consumption.

The raw AYB sample had significantly ($P<0.05$) higher (5.1 mg/g) phytate content compared to the treated samples. All AYB samples fermented in citric acid solution had significantly reduced phytate level that ranged from 2.2 mg/g in the whole AYB fermented for 24 h citric acid solution to 1.1 mg/g in dehulled AYB fermented for 48 h 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 to about 4.9 mg/g, brings about five folds increase in the bioavailability of iron [37]. The phytate levels of the products were reduced to about thrice this cited value. 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].

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
24 h 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
24 h 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
48 h 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
48 h 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
72 h 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
72 h 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 three replicates. Values are expressed as mean ± S.D. ^{a-n} values with different superscripts on the same column are significantly different (P=0.05)

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	24 h WAYB	24 h DAYB	48 h WAYB	48 h DAYB	72 h WAYB	72 h 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 three 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. 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 24 h (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 24 h (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 24 h(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 24 h (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 24 h(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 24 h(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 48 h (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 48 h (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 48 h(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 48 h (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 48 h(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 48 h (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 72 h(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 72 h (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 72 h (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 72 h (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 72 h (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 72 h (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}

*Values are mean of 10 panellists response on a 9-point hedonic scale. ^{a-d} values with different superscripts on the same column are significantly different (P=.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. 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 24 h	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
Dehulled AYB fermented for 24 h	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 24 hrs (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 24 h (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 48 h	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 48 h	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 48 h (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 48 h (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 72 h	4.44±1.18 ^d	4.83±1.21 ^e	4.67±1.12 ^c	7.01±0.04 ^a	4.00±0.62 ^d
Dehulled AYB fermented for 72 h	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 72 h (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 72 h (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

*Values are mean of 30 panellists response on a 9-point hedonic scale. ^{a-d} values with different superscripts on the same column are significantly different (P=.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

The oxalate level of the samples followed similar trends with other anti-nutrients as the raw samples had significant ($P<0.05$) the highest oxalate level of 0.21 mg/g, while the samples fermented for 72 h had significantly ($P<0.05$) the lowest oxalate level of 0.01 mg/g. The levels of oxalates in both fermented AYB and raw were low [41], therefore are safe for consumption because the lethal dose of oxalate is levels above 100 mg/100 g [42]. High oxalates in food cause irritation in the mouth or interfere with iron or calcium absorption [43]. The levels of oxalate obtained in this study could not 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 ranged from 0.3 mg/100 g in the raw sample to (0.00 mg/100 g) in 48h and 72h fermented samples. The saponin levels obtained in all the samples were low and within safe levels. A study on the lethal dose of saponin reported to be 200 mg/kg [44]. All the studied flours could be regarded as safe for consumption and the products would not exert negative effects like hemorrhage and erosion of the mucosa of the small intestine or necrosis of liver cells and renal tubules that has been attributed to the consumption of saponins [45].

Tannins were also significantly ($P<0.05$) reduced in all the processed samples hence, the reduction varied from 0.9 mg/g in raw AYB to 0.01 mg/g in dehulled AYB fermented for 72 h in 0.5% citric acid. The concentration of raffi nose 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 72 h. 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 acceptability for such foods [45,46]. Tannin levels in the range of 0.02 mg/g-0.05 mg/g were low which suggests 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 24 h and 48 h fermented samples. Dehulled AYB fermented for 72 h had the least level (0.01%) of stachyose in all the samples. The significant reduction in stachyose and raffinose in all the processed samples compared to 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 duration 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/100 g in the raw sample to 4.56 Hu/100 g in dehulled AYB fermented in 0.5% citric for 72 h. Studies have shown that haemagglutinin was unstable to traditional processing like fermentation, soaking, cooking, germination among others and also that a processed legume will hardly exert any toxic effects associated with foods that contain hemagglutinin [50].

Table 3 presents the effect of different concentrations 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 24h with 1% citric acid had similar highest scores for aroma (7.40) compared to those samples that were fermented for 72 h; that had the least scores. This result could be attributed to the fermentation time and concentrations of citric acid. As the duration 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 72 h. On the other hand, dehulled AYB fermented for 24 h using 1% citric acid had significantly ($P<0.05$) the highest score for colour (7.99), while dehulled AYB fermented for 24h in 0.25% citric acid had the least score (5.26) for colour. This result is expected as International Food Information Council (IFIC) and Food and Drug Administration [51] reported 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 24 h with 0.5% citric acid had significantly highest scores (7.79, 7.81) compared to other treated samples. It was observed that higher concentration of this acid lead to sour taste in the product limiting their acceptability. There were no significant difference ($p>0.05$) in texture for all the samples. Gruel made from whole and dehulled AYB fermented for 24h in 0.5% citric acid was the most acceptable of all the samples, having the highest scores of 7.32 & 7.22, respectively followed by both AYB fermented for 24 h in 1% citric acid solution.

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 24 hrs 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 72 hrs 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].

4. CONCLUSION

The findings from this study have shown that fermenting AYB seeds in 0.5% citric acid for 24h would produce wholesome flours free from beany flavour and bitter taste. Additionally, these flours would enhance the utilization of AYB. African yam bean being an underutilized crop that fears extinction might be revived and value might be added to it in various ways. The gruels made from flours fermented in 0.5% citric acid for 24 h has shown to have a considerable acceptability compared to gruels 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 anti-nutrients, the AYB fermented in 0.5% citric acid for 24h is recommended because of its higher protein, dietary fibre and better sensory quality.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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