

Original Research Article

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

ABSTRACT

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

Methodology: Cream coloured AYB seeds were purchased in Enugu, Enugu State Nigeria, in the month of December. Sorted AYB seeds were washed and fermented in citric acid medium (0.25%, 0.5% and 1%) for 24 hrs, 48 hrs and 72 hrs 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 hrs, 48 hrs and 72 hrs. At the end of the After fermentation, each batch of the fermented seeds were was divided into two. The first half were dehusked and the other half were left as whole. The fermented seeds were separately dried and milled into flour for further use. Standard laboratory methods were used for proximate, mineral and anti-nutrient analyses. Gruels were produced from all the flours and subjected to sensory evaluations using the a nine-point hedonic scale. Data generated obtained 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 $P < 0.05$.

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Results: Chemical compositions of the flour from seeds 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 whole raw and dehulled AYB seeds fermented for 24 hrs 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 24 hrs in 1% citric acid had the best highest score (7.99) for colour. Based on the sensory evaluation results, gruels made from the AYB seeds fermented in 0.5% citric acid was compared with the gruels made from AYB seeds that were fermented in tap water, and the results showed that gruels made from the AYB seeds that were fermented in 0.5% citric acid for 24 hrs had significantly better higher score for aroma (7.70), colour (7.10), and general overall acceptability (7.52). Food use Utilization of AYB in food formulation could be improved by fermenting in 0.5% citric acid solution for 24 hrs. Report the gruel with highest overall acceptability clearly.

Keyword: Citric acid, Fermentation, Proximate, Anti-nutrient, Sensory evaluation, Underutilized legumes, Gruel.

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1. INTRODUCTION

47 Food and nutrition insecurity, which arises from limited access to both qualitative and quantitative food to
48 meet dietary needs and food preferences are recurrent problems in Nigeria [1]. The food and nutrition
49 insecurity situation analysis conducted in March 2018 by the United Nation (UN) agencies in Nigeria
50 revealed that about 3.7 million Nigerians are facing food insecurity [2]. About 1 million Nigerians are in
51 emergency food situation and immediate intervention might be needed [3]. Food utilization has been cited
52 as one of the causes of food and nutrition insecurity. It is not enough to have access to food, but the food
53 should be free of secondary metabolites that antagonize their absorption and utilization. In achieving
54 good nutrition and sustainable food security, the use of indigenous ~~raw~~ food materials has been proposed
55 [4]. This is because ~~knowledge awareness creation and on use utilization~~ of indigenous foods ~~could~~ help
56 to improve nutritional status ~~of the consumers~~ and add variety to ~~their~~ diets [5]. Eating ~~right~~ nutritious food
57 is essential to achieving good health. ~~The knowledge of the best way to process and prepare food is~~
58 ~~essential to achieving proper utilization of the nutrients in food and consequently good health and~~
59 ~~nutrition.~~

60 ~~Among the many underutilized indigenous foods in Nigeria, the~~ Africa Yam bean (AYB) (*Sphenostylis*
61 *stenocarpa*) has been cited as one of the ~~underutilized~~ crops that have the potential of meeting food and
62 nutrition security in a sustainable way because of its great potentials [6]. It is a legume grown primarily for
63 its dry seeds and is widely grown in most parts of Africa [7]. It is fried and eaten mostly as snacks or
64 boiled and eaten as pottage or made into puddings in the eastern parts of Nigeria. Besides its use as
65 food, it is also used for medicinal purposes. Studies have shown the potential of AYB in the prevention
66 and management of Diabetes mellitus [8,9] and blood pressure with no negative effect ~~in-on~~ the liver [10].
67 Nutritionally, AYB ~~rates has~~ higher amino acid profile than most legumes ~~in terms of amino acid profile~~
68 [11]. ~~Its~~The protein concentrate is reportedly used for the fortification of starchy foods; ~~but~~ despite all
69 these attributes, its ~~use utilization~~ is limited by ~~its~~ beany flavour, ~~and~~ bitter taste, long cooking time of
70 about 6 ~~hours~~ and flatulence inducing oligosaccharides [12, 13]

71 ~~In~~ this study, ~~value~~ has ~~adopted been added to AYB the~~ using of different concentrations of citric acid
72 to produce a wholesome flour free of beany flavour and bitter taste. The use of ~~the these~~ flours to
73 produce gruels ~~hasve solved minimized~~ the problem of longing cooking time of about 6 ~~h_ours~~ and
74 ~~reduced it to 5minutes.~~

75 2. MATERIALS AND METHODS

76 2.1 Preparation of African yam bean flour

77 ~~2.1~~ Cream coloured AYB seeds were purchased from Enugu in Enugu State, Nigeria. The seeds
78 were sorted to remove impurities and weighed. ~~From~~ ~~the~~ sorted AYB ~~seeds, (20 kg)~~ was weighed,
79 washed and fermented in citric acid medium (0.25%, 0.5% and 1%) for 24 ~~hrs~~, 48 ~~hrs~~ and 72 ~~hrs~~ at room
80 temperature (28 °C) in a seed to citric acid ratio ~~of~~ 1:4 (w/v). At the end of fermentation, AYB seeds were
81 washed, each batch was divided into two and one-half of the fermented portions was dehulled, while the
82 remaining were left as whole. They were all placed in separate compartments in an oven (model number,
83 manufacturer's name and location) ~~digital food dehydrator which works by circulating cool dry air and~~
84 ~~removing moisture through a vent and ensuring food nutrients are not denatured (40-70 °C).~~ A start up
85 ~~temperature was~~ set at 70 °C for 4 ~~hrs~~ to dry up excess water and prevent further fermentation, ~~then~~
86 ~~the~~ temperature was reduced to 40 °C for what duration 44hrs? ~~when~~ until constant weight was obtained.
87 ~~They~~ were milled into fine flours using (70 mm mesh screen), ~~and~~ stored in airtight containers and
88 refrigerated until needed for chemical analysis and product development.
89
90

91 2.2 Proximate analysis

92 The methods described by the Association of Official Analytical Chemist (AOAC) ~~in~~ 2012 [14] were used in
93 determining moisture, ash, total fat and crude protein content of the samples. Dietary fibre was carried
94 ~~out~~ determined by Prosky, Asp, Furda, DeVries, Schweizer and Harland [15] method as described by
95 AOAC Method 985.29. Carbohydrate was determined by difference [14]. Clarify do you mean AOAC
96 2010 or 2012?
97

98 2.2.1 Determination of moisture content

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99

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

107

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

109

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

111 W_2 = weight (g) of the sample after drying

112

113 2.2.2 Determination of ash content

114

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

119

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

120

121

122 2.2.3 Dietary fibre

123

124 Using the method as described by Prosky *et al.* [15], The samples were cooked at 100°C with heat
125 stable α -amylase to give gelatinization, hydrolysis and depolymerization of starch; they were incubated at
126 60°C with protease (to solubilize and depolymerize proteins) and amyl glucosidase (to hydrolyze starch
127 fragments to glucose); they were treated with four volumes of ethanol to precipitate soluble fibre and
128 remove depolymerized protein and glucose (from starch). The residue was then filtered and washed with
129 78% ethanol, it was also washed with 95% ethanol, and finally acetone. After the washing, the residue
130 was dried and weighed. One duplicate was analyzed for protein and the other was incubated at 525°C to
131 determine ash. The Total Dietary Fibre was obtained by weighting the filtered and dried residue. The
132 result was deducted from the weights of the protein and ash.

133 2.2.4 Determination of Crude Fat

134

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

147

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

148

149 2.2.5 Determination of crude protein

150

151 One gramme (1g) of the sample powder was weighed out into 50 ml Kjeldahl digestion flask. Some 20 ml
152 concentrated H₂SO₄, 1 tablet of Kjeldahl catalyst and anti-bombing chips were added. The mixture was
153 incinerated to gentle boiling on the digestion rack and then heated further for 3 hours. The digest was
154 removed, cooled, quantitatively transferred to a 100 ml volumetric flask and made up to mark. Erlenmeyer
155 flask containing 10 ml of the boric acid indicator solution was placed at the tip of the condenser extended
156 below the surface of the solution. Ten millilitres (10 ml) of the sample digest was introduced into the
157 sample tube and steam heated, 10 ml of 40% NaOH solution was added to the digest and the digest was
158 steamed and distilled into the boric acid-indicator solution, it changed to green. A blank determination was
159 also carried out alongside that of the sample except that 1 g sample was replaced with 1ml distilled water.
160 The crude protein content was calculated as follows:

161
162 **Protein (%) = (A-B) × N × 1.4007 × 6.25**

163
164 Where

165 A= volume (ml) of 0.2 N HCl used sample titration

166 B = volume (ml) of 0.2 N HCl used in blank titration

167 N= Normality of HCl

168 W = weight (g) of sample

169 14.007 = atomic weight of nitrogen

170 6.25 = the protein-nitrogen conversion factor (6.25 is not a protein nitrogen conversion factor, 5.7 or
171 5.8?) The methods of AOAC (2012) were not expected to explain in detail.

172 173 | **2.2.6 Calculation-Determination of available carbohydrate content**

174
175 | The available carbohydrate content of the samples was calculated by difference using the formula below
176 [16].

177
178 | Available Carbohydrate = 100 – (crude protein + lipid + ash + moisture+ dietary fibre).

179 180 | **2.3 Determination of antinutrients**

181 182 | **2.3.1 Phytate determination**

183 Phytate content of sample was determined by-followed a simple and and rapid colorimetric method as
184 described by Latta and Eskin [17].

185 Five grams (5g) of the milled sample was weighed into a 250 ml conical flask, 100 ml of 2.45 HCL-M HCL
186 was added, and extracted for 1 hour at room temperature (25 °C±28 °C) and centrifuged.
187 Supernatant was decanted, 1 ml of 2.4% extract supernatant extract was diluted to 25 ml with distilled
188 water. Ten milliliters (10mls) of diluted sample was passed through the AG1-X8 chloride anion exchange
189 column (0.5 g). Phytate was eluted with 0.7 M NaCl, 3 ml of 0.7 M eluent fraction was pipetted into 15 ml
190 conical test tubes, and mixed on a vortex mixer for 5 seconds, and centrifuged for 10 minutes.
191 Absorbance of supernatant was read at 500 nm using water to zero the spectrophotometer. Series of
192 sodium phytate dilutions were made from 5-40 µg phytate in distilled water. Three millimeters (3ml) of the
193 solution was pipetted into 100µl ml cornical flask. One millimeter (4ml) of Wade reagent was added
194 within 30 minutes of elution. It was mixed on a vortex mixer for 5 seconds and centrifuged for 10 min.utes.
195 The absorbance of the supernatant was read at 500 nm using water to zero the standardize the
196 spectrophotometer. Phytate content was estimated from the standard curve.

197 198 | **2.3.2 Determination of trypsin inhibitors**

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

200 One hundred milligrams (0.04g) of sample was weighed; 20 ml cold (4 °C) methanol was added. It was
201 vortexed and centrifuged at 3,000rpm for 20 minutes.

202 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 0.5 ml
203 of estannic acid standard solution was pipetted into test tubes and made up to 5 ml by adding 5, 4.95,

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204 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
205 ppm.) ~~0.3 ml~~ From Folin-Denis reagent, 0.3 ml was added, 0.6 ml of Na₂CO₃ solution was also added.
206 The solution stood for 25-30 mins. The absorbance of blue color was read at 760 nm.

207 % Tannins was calculated from standard curve as follows:

208 $\%T_{\text{tannins}} = \frac{(A-I) \times V \times 100 \times D}{F}$

209 $B \times W \times 10^6$

210 A= Absorbance of sample.

211 I= Intercept

212 V= Total volume of extract

213 B= Slope of standard curve

214 W= Weight of sample

215 D.F = Dilution factor

216

217 2.3.3 Oxalic acid determination

218 Oxalate content was determined by following the method of Oke [19]. One gram (~~1g~~) of the flour
219 was extracted thrice by warming it at 40-50 ~~°C degree centigrade in a water bath (model number,~~
220 ~~manufacturer's name and location should be stated)~~ with constant stirring with using magnetic stirrer for 1
221 ~~hour~~ with 20 ml of 0.3 N HCl. The extract was diluted to 100 ml with distilled water. ~~5 ml~~ Five milliliters
222 of the extract was made alkaline with 1 ml of 5 N NH₄OH. This was made acidic with glacial acetic acid and
223 2 drops of phenolphthalein ~~servd was added~~ as an indicator (~~2 drops~~). 1 ml of 5% calcium chloride was
224 added and the mixture could stand ~~was rested~~ for 3 ~~hours~~, centrifuged using (IEC Centra GP8) at 1400
225 rpm for 15 min. ~~utes~~.

226 The supernatant was discarded, ~~and~~ the precipitate was washed thrice with hot water, mixed thoroughly,
227 mixing and centrifuged each time. Thereafter, 0.2 ml of 3 N H₂SO₄ was pipetted to each test tube
228 and ~~The~~ precipitate was dissolved by warming in water bath at 70 °C for 30 mins. The content of each
229 test tube was titrated with freshly prepared 0.01 N Potassium permanganate solutions. Titration was done
230 at room temperature (29 °C) until the colour of the solution become pink. The solution was allowed to
231 stand until it became colourless. It was warmed at 70 ~~degrees~~ °C and titrated until a pink color persisted
232 for 30 ~~seconds~~.

233 Calculations = oxalate content = $W \times 100/5$ (Clarify this)

234 W= Mass of oxalate in 100 ml

235 2.3.4 Determination of saponins

236 Saponins determination was carried out by following Fenwick and Oakenfull [20] procedure with
237 modification. The sample was finely ground and dried at constant weight. From dried sample, 40 g were
238 was weighed and placed in ~~the~~ Soxhlet reflux extractor with acetone for 24 ~~hours~~. The solvent was
239 changed for to methanol and extraction was continued for another 24 ~~hours~~. Why? The methanolic
240 extract was cooled and made up to 250 ml with methanol. At this point there was a This was
241 modification to the method by concentrating the sample, proposed by Miriam Monforte (CICY, Merida,
242 Yucatan, Mexico). Instead of bringing the sample up to 250 ml as suggested in the original method, it was
243 concentrated. In this, The methanolic extract was transferred into a rotary evaporator and concentrated
244 until dry. The residue was concentrated again in a minimum of methanol and transferred to a reweighed
245 vial. The vial was weighed with the dry sample and the weight of the residue was calculated. Fine drops
246 of a standard solution of saponins were placed on the chromatography plates. The points of extract were
247 placed so that each one is at the side of standard saponins drops. The plates were revealed? and the
248 drops with aspersion and a solution of sulphuric acid in methanol, it heated at 110°C for 30 min. (Clarify
249 this sentence). The intensity of the saponins stains was measured with a densitometer and the peak
250 areas were calculated on the plotter with a planimeter. The results were expressed as the relation (R) of
251 the peak areas of the unknown sample in respect to those that of the standard. R² was plotted against
252 the volume of the drop of methanolized extract on the plate. The downslope of the line (was calculated by

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253 | the least squares method), divided by the gradient of a line derived from a master standard curve, to give
254 | the concentration of saponins in the extract and thus, the saponins content of the sample.

255

256 | **2.3.5 Determination of lectins by spectrometric method**

257 | Lectins was determined according to the method described by Brooks [21]. Two grammes (~~2g~~) of
258 | the sample were weighed into 40 ml normal saline solution buffered at ~~P^H~~ pH 6.4 with 0.01M~~m~~ phosphate
259 | buffer solution. It ~~could was stand rested~~ at room temperature for 30 mins and centrifuged to obtain the
260 | extract. Half of a milliliter (~~0.5ml~~) of the extract was diluted in a test tube, 1 ml of heparinized rabbit blood
261 | was poured. The blank was prepared by adding 1 ml of the blood into a test tube and allowed to stand for
262 | 4 h at room temperature. ~~4ml of From~~ normal saline, 1 ml was added to all the test tubes and ~~it could~~
263 | ~~stand rested~~ for 10 min, after which the absorbance was read at 620 nm.

264 | Lectin unit/g= (b-a) x F- Use equation editor.

265 | Where b = absorbance of the blank

266 | F= experimental factor given by

267 | $F = (1/w \times f/va) D$

268 | Where

269 | W= weight of sample

270 | VF= total volume of extract

271 | VA= volume of extract used in the assay

272 | D= dilution factor

273 | Define all the parameters clearly for instance, the letters 'a' and 'v'.

274 | **2.3.6 Raffinose and stachyose determination**

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

277 | Five grams each of both raw and processed flour were extracted with 50 ml of 70% (v/v) aqueous
278 | ethanol and kept on an orbital shaker at 130 rpm for 13 h. Extracts were further washed with 25 ml of
279 | 70% (v/v) ethanol. The filtrates obtained were then concentrated on a water bath at what temperature and
280 | time?. The concentrated sugar syrup was dissolved in 5 ml of distilled water. Separation of
281 | oligosaccharides was done by Thin Layer Chromatography (TLC). A 100 g silica gel was dissolved in
282 | distilled water and stirred well until the slurry was homogeneous. The TLC plates were washed, dried and
283 | cleaned with chloroform to remove any grease from the plates. TLC plates were then coated with the
284 | slurry and air-dried. Spotting of the sugar samples was done by using capillary tubes. Each sample was
285 | spotted twice separately and dried using electronic hand drier (model number, name of the drier,
286 | manufacturer name and location of the manufacture are required). The plate was developed by using a
287 | solvent system of n-propanol, ethyl acetate and distilled water (6:1:3), and dried. The separated sugars'
288 | colours were developed with iodine crystals. The separated spots were compared with the standard sugar
289 | spots. The separated sugars that appeared were stachyose, raffinose and sucrose. The stachyose and
290 | raffinose spots were scrapped, eluted in 2 ml of distilled water, kept overnight and filtered through
291 | Whatman No.1 filter paper. The filtrates were measured and then subjected to quantitative estimation.
292 | ~~†~~The eluted individual oligosaccharide was estimated. One milliliter of the eluted and filtered sugar
293 | solution ~~was were~~ treated with one ml of concentrated HCl. The tubes were boiled in water bath for
294 | exactly 6 min at what temperature. After cooling, the absorbances of the oligosaccharide contents were
295 | read using spectrophotometer (name and model number of the equipment are required) 259 at 432 nm.
296 | The absorbance values were used to calculate the concentration and mass of the oligosaccharides.
297 | Average values of duplicate estimations were calculated, and the oligosaccharide contents expressed on
298 | a dry weight basis.

299

300

301 | **2.4 Gruel production Preparation of gruel**

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303 Gruels were prepared from the processed AYB flour (whole and dehulled) as described by
304 (standard method)

306 Recipe for gruel

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

307 Method of preparation

- 308 i. Seventy grams ~~(70g)~~ of tap water was used to reconstitute the flour
- 309 ii. Water (259 ml~~s~~) ~~was~~ brought to ~~the~~ boil.
- 310 iii. The boiled water was gradually added to the reconstituted flour, while stirring continuously to avoid
311 the formation of lumps.
- 312 iv. The mixture was returned to the pot, placed on fire and allowed to simmer gently
- 313 v. The mixture was simmered for 3 min~~utes~~ stirring continuously till cooked.
- 314 vi. Artificial sweetener was added and stirred (name of sweetner and quantity required)
- 315 vii. It was served hot

316 Method of preparation should be re-casted (in a continuous form not in an itemised form as above)

320 2.5 Sensory evaluation

322 In combination with an affective test (which one), multiple comparison tests (specify) were done,
323 using trained panelist at the preliminary stage to screen treatments and select the best concentration of
324 citric acid, fermentation time and ~~for~~ ratio of flour to use for final product development. After the products
325 were developed, ~~c~~Consumer acceptability of the products ~~were was~~ assessed using the semi-trained or
326 trained panellists to rate the samples affective test based on individual acceptability or preferences. It also
327 involves the rating/acceptance of the sample using a 9-point hedonic scale to determine the degree of
328 acceptability of the new products [23,24]

329 2.6 Statistical aAnalysis

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

335

336

337 3. RESULTS AND DISCUSSION

338

339 Effect of varying fermentation time on the proximate composition of whole and dehulled AYB 340 flours fermented in 0.05% citric acid solution (% dry weight basis).

341 Table 1 showed the effect of varying fermentation time on the proximate composition of treated
342 (0.5% citric acid) whole and dehulled AYB flours. The moisture content (10.2-10.5%) of the raw and 24 h~~f~~
343 fermented whole and dehulled AYB were statistically not significantly ($P > 0.05$) similar different, but differ
344 significantly ($P < 0.05$) from those fermented for 48 and 72 hours. The moisture contents of the processed

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345 flours were all within the safe levels for storage [25]. The moisture content of food is a good indicator for
 346 ensuring its keeping quality. Dehulled AYB fermented in citric acid solution for 24_hrs had the highest
 347 protein (30.5%). The 24_h and 72_h whole and 72_hf dehulled AYB fermented in citric acid had similar
 348 protein values (26.9%, 26.3% and 27.1% respectively). Raw untreated AYB had the least protein value
 349 (20%). The significant increase observed in the protein content of the flours especially dehulled AYB
 350 fermented for 24_hrs in 0.5% citric acid medium is not surprising-far fetched as studies have indicated an
 351 increase in the protein contents of fermented foods [26, 27]. The result can also be attributed to the citric
 352 acid used; fermentation in citric acid medium have been shown to significantly increase the protein
 353 contents of foods, this increase can be attributed to the low pH provided by the citric acid thereby
 354 facilitating protein solubilization, resulting in higher protein yields [28,29]. The length of fermentation and
 355 dehulling might have contributed to the protein increase because the fermentation time (24_hrs) might not
 356 have permitted leaching of the proteins in the fermentation medium and also because proteins are more
 357 concentrated in the cotyledon, therefore dehulling reduces the bulking effect and makes the proteins
 358 concentration of the dehulled samples higher [30]. The fat contents significantly (P<0.05) decreased with
 359 the treatment. Raw untreated-AYB had 1.6% fat, while whole AYB fermented for 72_hf had 1% fat. The
 360 decreases in the fat content of the AYB flour samples are in line with several studies that reported
 361 decreases in fat content in fermented products [31], the low-fat content of the processed flours further
 362 enhance the keeping quality of the flour as rancidity will be reduced [32]. The ash contents also
 363 decreased with processing even though the~~re~~ were slight variations. They ranged from 2.3% in dehulled
 364 AYB fermented for 72_hrs to 2.7% in raw AYB. Ash content indicates the level of mineral element [33].
 365 The dietary fibre values varied significantly (P<0.05) from (14.3 to 17.5%). There was a decrease in
 366 dietary fibre with processing from 17.5% in raw AYB to 14.3% in dehulled AYB fermented for 72_hrs. The
 367 reduction in the dietary fibre of all the AYB flours especially the dehulled sample was expected as more
 368 dietary fibre will be expected in the whole AYB sample and studies have reported reduction in the fibre
 369 contents of fermented legumes [34]. All the fermented samples had significant reduction in their
 370 carbohydrate contents; this was expected with the increase in protein and in some cases fat.

371

372

373

TABLE 1

374 Effect of varying fermentation time on the proximate composition of treated (0.5% citric acid)
 375 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
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376 *Means of three replicates. Values are expressed as mean ± S.D. ^{a-h} values with different superscripts on the same column
377 are significantly different ($P \leq 0.05$).

378

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

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

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

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390 The raw AYB sample had significantly ($P < 0.05$) higher (5.1 mg/g) phytate content compared to
391 the treated samples. All AYB samples fermented in citric acid solution had significantly reduced phytate
392 level ~~and they that~~ ranged from 2.2 mg/g in the whole AYB fermented for 24 hrs citric acid solution to 1.1
393 mg/g in dehulled AYB fermented for 48 hrs in citric acid solution. Phytate was reduced to safe levels in all
394 the fermented samples. Studies have shown that reduction of phytate levels in food ~~are reduced~~ to about
395 4.9 mg/g, brings about five folds increase in the bioavailability of iron [37]. The phytate levels of the
396 products were reduced to about ~~three timesthrice~~ this cited ~~valuelow level~~. Also, the phytate-zinc molar
397 ratios observed from this study were very low indicating a high bioavailability of zinc. Therefore, the
398 phytate level of the flours and their products might be incapable of chelating calcium or limiting the
399 bioavailability of iron or zinc [38,39]. Citric acid treatment of AYB must have contributed to the reduction
400 of phytic acid as it lowered the pH of the fermented flour; and studies have shown that phytic acid
401 reduction is aided by low pH [40].

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402 The oxalate level of the samples followed similar trends with other anti-nutrients as the raw
403 samples had significant ($P < 0.05$) the highest oxalate level of 0.21 mg/g, while the samples fermented for
404 72 hrs had significantly ($P < 0.05$) the lowest oxalate level of 0.01 mg/g. The levels of oxalates in both
405 fermented AYB and ~~com raw~~ were low [41], therefore ~~can beare~~ safe ~~for consumption~~ because the
406 lethal dose of oxalate is levels above 100 mg/100 g [42]. High oxalates in food cause irritation in the
407 mouth or interfere with iron or calcium absorption [43]. The levels of oxalate ~~observed-obtained~~ in this
408 study ~~are could not likely to either~~ interfere with iron or calcium absorption or lead to the formation of
409 stones in the urinary tract.

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410 Saponins were significantly reduced in all the processed samples. The reduction ~~was ranged~~
411 from 0.3 mg/100 g in the raw sample to ~~trace~~ (0.00 mg/100g) in 48 hrs and 72 hrs fermented samples.
412 The saponin levels ~~observed-obtained~~ in all the samples were low and within safe levels. A study on the
413 lethal dose of saponin was ~~observed reported~~ to be 200 mg/kg [44]. All the studied flours could be
414 regarded as safe for consumption and the products ~~might would~~ not ~~exact exert~~ negative effects like
415 hemorrhage and erosion of the mucosa of the small intestine or necrosis of liver cells and renal tubules
416 ~~that was has been~~ attributed ~~able~~ to the consumption of saponins [45].

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417 Tannins were also significantly ($P < 0.05$) reduced in all the processed samples hence, the
418 reduction ~~was varied~~ from 0.9 mg/g in raw AYB to 0.01 mg/g in dehulled AYB fermented for 72 hrs in
419 0.5% citric acid. The concentration of raffinose was significantly ($P < 0.05$) reduced with processing. It was
420 higher (2.18%) in the raw sample than the samples fermented in citric acid solution, but significantly
421 ($P < 0.05$) lowest (1.38%) in dehulled AYB fermented for 72 hrs. The significant reduction in the tannin
422 contents of the processed samples might have contributed to the acceptability of the products as products
423 with high tannin levels are known to have bitter taste thus, reducing consumer ~~choice-acceptability~~
424 for such foods [45, 46]. Tannin levels in the range of 0.02 mg/g – 0.05 mg/g ~~are regarded as were~~ low ~~which~~

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425 | suggesting that these products might not form complex with protein, starch, cellulose or minerals
 426 | because of the significant reduction of the tannin content [47].

427 | Fermentation in citric acid medium had significant ($P<0.05$) impact by reducing the stachyose
 428 | contents of the treated samples in comparison with the raw sample. The raw AYB (3.16%) had
 429 | significantly ($P<0.05$) higher stachyose than all other samples. There was no significant difference in the
 430 | level of stachyose in 24_hrs and 48_hrs fermented samples. Dehulled AYB fermented for 72_hrs had the
 431 | least level (0.01%) of stachyose than in all other the samples. The significant reduction in stachyose and
 432 | raffinose in all the processed samples, when compared with to the raw AYB is in line with a study by
 433 | Chen [48] that reported a similar reduction in the oligosaccharide content of fermented soybean. This
 434 | reduction is an indication that the diets might have less toxicological and nutritional problems like
 435 | diarrhea, gas production with belching, flatulence, abdominal bloating and pain [49]. From the study,
 436 | fermentation duration time influenced stachyose and raffinose reduction, that is, the longer the
 437 | fermentation time, the more reduced the oligosaccharide content. The significant reduction of
 438 | haemagglutinin in all the samples could be attributed to fermentation. Haemagglutinin was significantly
 439 | reduced from 32 Hu/100_g in the raw sample to 4.56 Hu/100_g in dehulled AYB fermented in 0.5% citric
 440 | for 72_hr. Studies have found-shown that haemagglutinin to be most was unstable to traditional processing
 441 | like fermentation, soaking, cooking, germination among others and also that a processed legume will
 442 | hardly exert any toxic effects associated with foods that contain hemagglutinin [50]. Other studies have
 443 | stated that there is no evidence that hemagglutinin in food will have any toxic effect after cooking
 444 | (reference required).
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446 | TABLE 2

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

	Raw AYB	24_hrs WAYB	24_hrs DAYB	48_hrs WAYB	48_hrs DAYB	72_hrs WAYB	72_hrs DAYB
Trypsin inhibitor s (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/100g)	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}
Raffinos e (%)	2.18 ± 0.02 ^a	1.76 ± 0.2 ^b	1.56 ± 0.01 ^c	1.76 ± 0.01 ^a	1.56 ± 0.02 ^c	1.58 ± 0.01 ^c	1.38 ± 0.01 ^c
Stachy se	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/100g)	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

449 | DAYB- Dehulled AYB Flour, WAYB- Whole AYB Flour.
 450 | *Values are Means-means of three3 replicates. Values and are expressed as mean ± S.D. ** values with different
 451 | superscripts on the same row are significantly different ($P<=0.05$).
 452 |

453 |
 454 | Table 3 presents the effect of different concentrations of citric acid (0.25%, 0.5% & 1%) and varying
 455 | fermentation time on the sensory characteristics of whole and dehulled AYB gruel. Whole and dehulled
 456 | AYB fermented for 24_hrs with 0.51% citric acid had similar significantly ($P< 0.05$) highest scores for

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457 | aroma (7.430, 7.35, respectively) compared to those samples that were fermented for 48hrs and 72 hrs;
 458 | with samples fermented at 72hrs that having had the least scores. This result can-could be attributed to
 459 | the fermentation time and concentrations of citric acid. As the length-duration of fermentation increases,
 460 | the score for aroma decreases. This is evident in the least score for aroma observed in the samples that
 461 | were fermented for 72 hrs. On the other hand, dehulled AYB fermented for 24_h using 1% citric acid had
 462 | significantly ($P<0.05$) the highest score for colour (7.99), while dehulled AYB fermented for 24_hrs in
 463 | 0.25% citric acid had the least score (5.26) for colour. This result is not-surprisingexpected as
 464 | International Food Information Council (IFIC) and Food and Drug Administration [51] reportednoted that
 465 | citric acid can be used to improve colour, more so, the improved colour in the sample with the highest
 466 | concentration of citric acid (1%) could be linked to the concentration. In terms of taste, AYB fermented for
 467 | 24_hrs with 0.5% citric acid had a significantly highest scores (7.79, 7.81) than all-compared to other
 468 | treated samples. It was observed that higher concentration of this acid lead to sour taste in the product
 469 | limiting their acceptability. There were No-no significant difference ($p>0.05$) was observed for in texture
 470 | for all the samples. Gruel made from whole and dehulled AYB fermented for 24_hrs in 0.5% citric acid was
 471 | the most acceptable of all the samples, having a-significantlythe highest scores of 7.32 & 7.22,
 472 | respectively, followed by both AYB fermented for 24_hrs in 1% citric acid solution.

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

Samples	Aroma	Colour	Taste	Texture	Overall General acceptability
Whole AYB fermented for 24_hrs (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_hrs (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_hrs (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_hrs (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_hrs (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_hrs (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_hrs (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_hrs (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_hrs (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_hrs (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_hrs (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_hrs (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_hrs (0.25% citric acid)	4.77±1.10 ^e	5.40±1.84 [†]	4.40±1.85 ^d	7.02±1.28 ^a	4.50±1.10 ^d
Dehulled AYB fermented for 72_hrs (0.25% citric acid)	4.82±1.10 ^e	5.26±1.10 [†]	4.32±1.28 ^d	7.14±1.28 ^a	4.46±1.21 ^d
Whole AYB fermented for 72_hrs (0.5% citric acid)	4.80±1.28 ^e	5.40±1.28 [†]	5.40±1.56 ^c	6.98±1.58 ^a	4.94±1.15 ^d
Dehulled AYB fermented for 72_hrs	4.85±1.45 ^e	5.44±1.23 [†]	5.39±1.42 ^c	7.00±1.15 ^a	4.91±1.06 ^d

(0.5% citric acid)						
Whole AYB fermented for 72_hrs (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_hrs (1% citric acid)	4.95±1.33 ^e	5.83±1.12 ^{et}	5.67±0.90 ^c	7.01±1.15 ^a	5.00±1.14 ^{bd}	

478 *Values are mean of 10 panellists response on a 9-point hedonic scale. ^{a-d} values with different superscripts on the
479 same column are significantly different ($P \leq 0.05$). 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
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486 Table 4 presents the effect of varying fermentation time duration on the sensory characteristics of gruels from
487 whole and dehulled AYB fermented in citric acid solution and tap water.

488 When compared-Compared to other samples, gruels from whole and dehulled AYB fermented for 24_hrs in
489 0.5% citric acid solution had significantly ($P < 0.05$) highest scores for aroma (7.70, 7.65), colour (6.99, 7.10),
490 taste (7.78, 7.00) and general-overall acceptability (7.54, 7.23), respectively than all the samples that were not
491 treated with citric acid but were fermented in tap water. No-significant-Significant difference ($p > 0.05$) was not
492 observed for texture in all the tested samples. Gruels of whole and dehulled AYB fermented for 72_hrs in tap
493 water had the least value for aroma (4.44, 4.55), colour (4.83, 4.87) and general-overall acceptability status
494 (4.00, 3.99), respectively. The highest overall acceptability status-obtained-of-for the gruel made from AYB
495 that was fermented in 0.5% citric acid solution for 24_hrs could be as a result of the treatment with citric acid
496 and the concentration used, as citric acid is known to improve colour, aroma and enhance the taste of foods
497 [51, 52, 53].

498

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

	Aroma	Colour	Taste	Texture	<u>General-Overall</u> acceptability
Whole AYB fermented for 24_hrs	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_hrs	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_hrs (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_hrs	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

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Dehulled AYB fermented for 48 hrs	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 hrs (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 hrs (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 hrs	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 hrs	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 hrs (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 hrs (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

501 *Values are mean of 10 panellists response on a 9-point hedonic scale. ^{a-d}values with different superscripts on the same
502 column are significantly different (p<=0.05). Organoleptic Scores/rating 1=~~Dislike~~ extremely, 2=~~Dislike~~ very much, 3=~~Dislike~~
503 moderately, 4=~~Dislike~~ slightly, 5=~~Neither~~ like nor dislike, 6=~~Like~~ slightly, 7=~~Like~~ moderately, 8=~~Like~~ very much,
504 9=~~Like~~ extremely

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