1	Original Research Article
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3	CHANGES IN AMINO ACIDS, ANTI-NUTRIENTS AND
4	FUNCTIONAL PROPERTIES OF AFRICAN YAM BEAN FLOUR
5	CAUSED BY VARIATION IN STEEPING TIME PRIOR TO
7	ACTOCLATING
8	
9 10	
11	ABSTRACT
12	
13 14	Background and Aim
15	Legume code are usually steeped in water or biserbanate colution
12	Legume seeds are usually steeped in water of bicarbonate solution
16	before further processing and this has some effect on the constituents
17	and functional properties of the flour. The present study was to
18	evaluate the effect of steeping time in 0.50% solution of unripe
19	plantain peel ash prior to autoclaving on the amino acids, anti-
20	nutrients and functional properties of African yam bean flour.
21	
22	Study Design:
23	Analysis of variance (ANOVA) was carried out.
24	
25	Place and Duration:
26	Department of Food Science and Technology, University of Uyo, Akwa
27	Ibom State, Nigeria, from November 2016 to May 2017.
28	
29	Methodology:
30	African yam bean seeds were sorted, washed and divided into four
31	portions of 500g each. Portion I was not steeped and served as control
32	sample while portions 2, 3 and 4 were steeped in 0.50% solution of
33	unripe plantain peel ash (1:5w/v) at ambient temperature (27 \pm 2°C)
34	for 24, 48 and 72h respectively. Both the unsteeped and steeped

portions were separately autoclaved, dehulled, oven dried, milled andsieved to obtain the flours used for analysis.

37 **Result:**

38 The result revealed that all the parameters varied with steeping time. 39 The total amino acids and total essential amino acids increased from 40 75.52g/100g and 30.07g/100g in the flour from unsteeped seeds to 41 80.29g/100g and 32.87g/100g respectively in the flour from the seeds 42 that were steeped for 72h. Phytate, tannin, trypsin inhibitor, reffinose, 43 stachyose, bulk density, water absorption capacity and swelling index 44 decreased while oil absorption and foaming capacities increased with 45 steeping time. Percentage reduction of phytate, tannin, trypsin 46 inhibitor, raffinose and stachyose after 72h steeping were 80, 86, 98, 47 97 and 94%, respectively.

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49 **Conclusion:**

50 The treatment caused significant (p<0.05) reduction in anti-nutrients 51 and flatulence causing factors and enhanced amino acid profile of the 52 flour.

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54 Keywords:African yam bean flour, steeping time, amino acids,55anti-nutrients, functional properties.

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59 1. INTRODUCTION

Legumes are good sources of cheap and readily available protein for human consumption [1]. Due to their high protein content, legumes have been promoted as a source of protein especially for low income families in countries with high rates of protein – energy malnutrition and the usage of animal protein is an economic constraint. They are also excellent sources of 66 carbohydrate, fairly good sources of minerals and vitamins [2, 3] as well as health protecting bioactive compounds ([4, 5]. 67 However, only a few of the known legume species are 68 69 extensively promoted and use in Nigeria while the rest are 70 underexploited and underutilized. Studies have shown that 71 lesser known legumes together with conventional legumes can 72 be used for combating protein - energy malnutrition in the developing countries [6]. 73

74 African Yam Bean (AYB) is among the lesser known 75 legumes having the potential to reduce protein deficiency in 76 developing nations. It is a climbing legume with an exceptional 77 ability for adaptation to low land tropical conditions [7]. In 78 Nigeria, the beans are cultivated by subsistence farmers for 79 their seeds although there are reports about the use of tubers as 80 food [8]. The protein contents in the seeds range from 15.5% to 81 34.7% with a fairly good source of amino acids [7, 8]. Amino 82 acid analysis indicated that the level of lysine and methionine in the seed protein are equal to or better than those of soybean 83 84 [9]. This implies that the seed could be used to supplement 85 cereal proteins which are deficient in lysine. The seeds are also 86 rich in mineral elements including potassium, calcium, phosphorus, magnesium, iron and zinc but low in sodium 87 content [9]. 88

89 Despite the nutritional potentials of African yam bean, its 90 utilization has been limited because of the hard-to-cook 91 characteristics which lead to high fuel requirement, possession of objectionable beany flavour, and problem of flatulence upon 92 93 consumption and the presence of anti-nutritional substances. 94 Trypsin inhibitors, phytic acid, tannin, saponin and oxalate are 95 among the anti-nutrients present in African yam bean seeds 96 [10, 11, 12]. These anti-nutrients hinder the efficient utilization, 97 absorption or digestion of some nutrients and thus reduce their 98 bioavailability and their nutritional value. Attempt to increase 99 the utilization of legumes have employed a wide range of 100 processing techniques such as soaking, boiling, sprouting, 101 fermentation, autoclaving and toasting. Shimelis et al. [13] 102 however noted that no single method can effectively eliminate 103 most the toxic factors in legumes and therefore suggested a 104 combination of methods to remove them. These processing 105 techniques may also have positive or negative effects on other constituents in the seeds and functional properties of the flour. 106

107 Traditionally, cereals and legumes are prepared in 108 common household by soaking or steeping in water or alkaline 109 solution followed by cooking or other thermal treatment. 110 Steeping is often used as secondary process aid to most of 111 plant-based food preparations. Soaking has been reported to 112 reduce the cooking time of legumes [14], decrease the anti-

113 nutrients [15, 16] as well as concentration of stachyose and 114 raffinose which are related to flatulence problem [17, 18]. It 115 also affects the nutrient content [19, 20] and functional 116 properties of the flour prepared from the soaked seeds [21]. The 117 present study was conducted to assess the effect of varying the 118 duration of steeping African yam bean seeds in 0.50% solution of unripe plantain peel ash prior to autoclaving on the amino 119 acids content, anti-nutrients and functional properties of the 120 121 flours.

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123 2. MATERIALS AND METHODS

124 **2.1 Sample Procurement**

Brown coloured variety of African yam beans (*Sphenostylis* stenocarpa) used for this study were purchased from a local market in Ini Local Government Area of Akwa Ibom State, Nigeria and transported to the Department of Food Science and Technology Laboratory, University of Uyo, Nigeria for processing and analysis.

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132 2.2 Preparation of Unripe Plantain Peel Ash

133 The unripe plantain peels usually thrown away as waste 134 product were washed in potable water, drained, dried at 100°C 135 in a hot air oven (model pp. 22 US, Genlab, England) for 18 hr, 136 incinerated, cool in a desiccators, packaged in an air tight 137 container and stored at 4°C for subsequent use.

2.3 Processing of African Yam Bean Seeds into Flours

Immature and infected seeds as well as other unwanted 140 141 materials were carefully sorted out and discarded while the good 142 seeds were used for the study. Two kilograms (2kg) of the sorted seeds were washed in potable water, drained and shared 143 144 into four equal portions of 500 grams each. Portion 1 was not 145 steeped and served as the control sample while portions 2, 3 146 and 4 were separately steeped in 0.50% solution of unripe plantain peel ash (1:5w/v) in a plastic container and kept in a 147 148 dark room at ambient temperature $(27\pm2^{\circ}C)$ for 24, 48 and 72h 149 respectively. At the end of each steeping period, the solution was decanted, the seeds rinsed twice with potable water and 150 151 autoclaved at 121°C under 15 atmospheric pressure in distilled 152 water (1:3w/v) for 15 minutes. Unsteeped seeds were also 153 autoclaved under the same condition. The autoclaved seeds 154 were drained from the excess autoclaving solution, rinsed with water and manually decorticated. The decorticated seeds were 155 156 dried in an oven (model pp. 22 US, Genlab, England) at 60°C to 157 constant weight, winnowed, milled and sieved to pass through 158 450 micrometer mesh screen. Flour from each of the portions 159 was separately packaged in an air tight plastic container, labeled and stored at 4°C for various determinations. 160

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166 2.4 METHODS OF ANALYSIS

Amino acid profile of the samples was determined by the 167 168 method described by Spackman et al. [22]. The samples for 169 amino acid determination were dried to constant weight, 170 defatted, hydrolyzed, evaporated in a rotary evaporator (Labratorums Technic AG, Model CH - 9230) and loaded into a 171 172 Technicon Multi-Samle Amino Acid Acid Analyzer (TSM) 173 (Technicon TSM-1, Model DNA 0209, Dublin, Republic of 174 Ireland). pH of flours was measured using a digital pH meter 175 (JENWAY, PHS – 25, ALibata). Total titratable acidity and tannin were determined following the methods described in AOAC [23]. 176 177 The method described by Oberleas [24] was used for phytate 178 determination. Trypsin inhibitor analysis was done using 179 spectrophotometric method described by Arntfield [25]. Oligosaccharides (raffinose and stachyose) were determined 180 following the method described by Doss et al. [6]. The method 181 182 described by Okezie and Bello [26] was followed for the 183 determination of bulk density of the flours. Water absorption 184 capacity (WAC), oil absorption capacity (OAC), foaming capacity (FC) and swelling index (SI) were determined following the 185 methods described by Abbey and Ibeh [27]. 186

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188 2.5 Statistical Analysis

Data obtained (in triplicate) were subjected to One Way Analysis of Variance (ANOVA) using SPSS version 18 statistical package (SPSS, Inc., USA) software. Significant difference comparisons were made using the Duncan's Multiple Range Test (DMRT) at P = 0.05.

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195 **3. RESULTS**

196**3.1**Effect of Steeping Time Prior to Autoclaving on Amino197Acids Content in African yam Bean Flour

199 The effect of steeping time prior to autoclaving of African yam 200 bean seeds on the amino acid profile of the flour is presented on 201 Table 1. The result showed that the individual, total amino acids 202 and total essential amino acids in the flours varied with the steeping period. The total amino acids in the flour from 203 204 unsteeped but autoclaved seeds was 75.52g/100g protein while 205 the values for the flours from seeds that were steeped for 24, 48 206 and 72h prior to autoclaving increased to 79.15, 76.26 and 80.29g/100g protein respectively. Glutamic acid was the highest 207 contributor in the total amino acid content and was followed by 208 209 aspartic acid. The total essential amino acid for the flour from 210 unsteeped but autoclaved seed was 30.07g/100g protein. 211 Steeping the seeds for 24, 48 and 72h prior to autoclaving led to insignificant (p>0.05) increased in the total essential amino 212 213 acids to 32.22, 31.49 and 32.87g/100g protein respectively. 214 Leucine was the highest contributor of the total essential amino

acids and was followed by lysine. Histidine and methionine were
the two least essential amino acids in the flours from the
unsteeped and steeped seeds.

Table 1:	Effect of steeping time prior to autoclaving on			
	the amino acid content in African yam bean			
	flour (a/100a Protein)			

Amino Acids	Soaking Time (hrs)			
	0	24	48	72
Lysine	$5.30^{a} \pm 0.01$	5.56°±0.06	5.52 ^ª ±0.02	5.59 ^ª ±0.03
Histidine	2.04 ^a ±0.04	2.14 ^a ±0.02	2.11 ^ª ±0.01	2.16 ^a ±0.12
Arginine	$6.36^{b} \pm 0.01$	6.79 ^a ±0.01	6.59 ^a ±0.06	6.88 ^a ±0.05
Aspartic acid	8.74 ^a ±0.03	8.80 ^a ±0.03	8.65°±0.02	8.80 ^a ±0.03
Threonine	2.94 ^ª ±0.02	2.99 ^a ±0.01	2.92ª±0.03	3.15ª±0.10
Serine	3.13 ^ª ±0.01	3.32 ^a ±0.04	3.14 ^a ±0.01	3.40 ^a ±0.06
Glutamic acid	$11.51^{a} \pm 0.05$	11.66ª±0.02	10.90 ^b ±0.05	11.71 ^b ±0.02
Proline	3.35°±0.00	3.45 ^ª ±0.02	3.30 ^a ±0.02	3.58 ^ª ±0.01
Glycine	$3.70^{a} \pm 0.10$	3.80 ^a ±0.05	3.61 ^ª ±0.04	3.82 ^a ±0.02
Alanine	3.37 ^b ±0.03	3.75 ^ª ±0.03	$3.50^{b} \pm 0.10$	$3.80^{b} \pm 0.04$
Cystine	1.45°±0.04	1.45°±0.04	1.33ª±0.08	1.45°±0.06
Valine	4.38 ^b ±0.02	4.70 ^a ±0.10	4.64 ^a ±0.03	4.91 ^ª ±0.01
Methionine	1.01 ^ª ±0.03	1.20 ^a ±0.02	1.17 ^a ±0.01	1.23ª±0.05
Isoleucine	4.09 ^b ±0.12	4.55°±0.06	4.41 ^ª ±0.05	4.45 ^a ±0.00
Leucine	6.77 ^b ±0.05	7.18 ^a ±0.02	6.94 ^b ±0.03	7.30 ^b ±0.04
Tyrosine	2.92 ^ª ±0.02	2.92 ^a ±0.00	2.81 ^ª ±0.02	2.92 ^ª ±0.06
Tryptophan	0.92 ^ª ±0.02	0.99 ^a ±0.03	0.94 ^a ±0.11	1.06 ^a ±0.05
Phenylalanine	3.54 ^c ±0.04	3.90 ^a ±0.02	3.78 ^b ±0.02	4.08 ^b ±0.02
ТАА	75.52 ^b ±0.02	79.15°±0.01	76.26 ^b ±0.04	80.29 ^a ±0.03
TEAA	$30.07^{a} \pm 0.01$	32.22 ^ª ±0.02	31.49 ^ª ±0.01	32.87 ^a ±0.02

Values are means \pm SD (standard deviation) of triplicate determinations. Means on the same row with different superscripts are significantly different at P = 0.05. TAA = total amino acid; TEAA = total essential amino acid

230 231 3.2 Effect of Treatment on pH, Total Titratable Acidity and 232 Anti-Nutrients of AYB Flour

- Result on Table 2 shows that the pH and total titratable
 acidity of the flours were affected by the steeping time prior to
 autoclaving.
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Table 2: Effect of steeping time prior to autoclaving on
pH, titratable acidity and some anti-nutritional
factors in African yam bean flour

Parameters	Soaking Time (hrs)			
	0	24	48	72
рН	5.53 ^b ±0.00	$5.81^{a} \pm 0.00$	$5.58^{b} \pm 0.00$	5.34 ^a ±0.00
TTA (%)	0.13 ^c ±0.05	$0.10^{\circ} \pm 0.11$	$0.18^{b} \pm 0.20$	$0.23^{a} \pm 0.04$
Phytate (mg/100g)	$8.60^{b} \pm 1.2$	$5.21^{b} \pm 0.09$	2.97 ^c ±0.11	$1.65^{a} \pm 0.08$
	(0.00)	(39.42)	(65.47)	(80.81)
Tannin (mg/100g)	7.90 ^ª ±0.30	$5.36^{b} \pm 0.75$	$2.14^{c} \pm 0.40$	$1.10^{a} \pm 0.15$
	(0.00)	(32.15)	(72.91)	(86.08)
TIA (TIU/mg)	$18.72^{a} \pm 0.51$	$10.45^{b} \pm 0.60$	4.96 ^c ±0.13	$0.24^{a} \pm 0.32$
	(0.00)	(44.18)	(73.50)	(98.72)
Raffinose (%)	0.43 ^ª ±0.09	$0.14^{b} \pm 0.03$	$0.07^{c} \pm 0.12$	$0.02^{a} \pm 0.05$
	(0.00)	(67.44)	(83.72)	(95.35)
Stachyose (%)	$0.51^{a} \pm 0.04$	$0.16^{b} \pm 0.10$	$0.10^{c} \pm 0.08$	$0.04^{b} \pm 0.20$
	(0.00)	(68.63)	(80.39)	(92.17)

241Values are means \pm SD (standard deviation) of triplicate242determinations. Means on the same row with different243superscripts are significantly different at P = 0.05. TTA = total244titratable acidity; TIA = trypsin inhibitor activity. Values in245parenthesis indicate percent reduction

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The pH and total titratable acidity of the flour prepared from unsteeped but autoclaved seeds were 5.53 and 0.13%, respectively. Steeping the seeds for 24h led to increase in pH to 5.81 and a decrease in total titratable acidity to 0.10. Prolonging the steeping time to 48 and 72h resulted in decreased in pH while the total titratable acidity significantly (p<0.05) increased. The results on Table 2 also show that phytate, tannin, trypsin inhibitors, raffinose and stachyose significantly (p<0.05) decreased from 8.60mg/100g, 7.90mg/100g, 18.72TIU/mg, 0.43% and 0.51% in the flour from unsteeped but autoclaved seeds to 1.65mg/100g, 1.10mg/100g, 0.24TIU/mg, 0.01% and 0.03%, respectively in the flour from 72h steeped and autoclaved seeds.

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- **3.3 Effect of the Treatment on Functional Properties of AYB** Flour

265 Data on Table 3 depicts the effect of steeping time prior to 266 autoclaving on some functional properties of African yam bean 267 flour.

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Table 3: Effect of steeping time prior to autoclaving on functional properties of African yam bean flour

Parameters		Soaking Time (hrs)			
		0	24	48	72
	Bulk density (g/cm ³)	0.67 ^a ±0.01	0.64 ^a ±0.05	0.63 ^a ±0.02	0.61 ^a ±0.04
	OAC (ml/g)	0.75 ^a ±0.09	0.78 ^a ±0.12	0.77 ^a ±0.06	0.79 ^a ±0.11
	WAC (ml/g)	1.82 ^a ±0.14	1.75 ^b ±0.08	1.71 ^b ±0.03	1.69 ^b ±0.05
	Foaming capacity (%)	2.65 ^b ±0.06	3.82 ^a ±0.04	3.86 ^a ±0.10	3.92 ^a ±0.02
	Swelling index (ml/g)	1.58 ^a ±0.03	1.44 ^a ±0.02	1.32 ^a ±0.05	$1.21^{b} \pm 0.08$

271Values are means \pm SD (standard deviation) of triplicate272determinations. Means on the same row with different273superscripts are significantly different at P = 0.05. OAC = oil274absorption capacity; WAC = water absorption capacity.

276 The result showed that the bulk density, water absorption 277 capacity (WAC) and swelling index progressively decreased 278 while oil absorption capacity (OAC) and foaming capacity increased with longer steeping time. The bulk density, water 279 280 absorption capacity and swelling index decreased from 281 0.67g/cm³, 1.82ml/g and 1.58ml/g for the flour prepared from unsteeped but autoclaved seeds to 0.61g/cm³, 1.69ml/g and 282 1.21ml/g, respectively for the flour from seeds that were 283 steeped for 72h prior to autoclaving. Conversely, the oil 284 absorption capacity and foaming capacity increased from 285 286 0.75ml/g and 2.65% for the flour prepared from unsteeped seeds to 0.79ml/g and 3.92%, respectively for the flour from 287 288 the seeds that were steeped for 72h prior to autoclaving.

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290 4. **DISCUSSION**

291 **4.1 Effect of Treatment on Amino Acid Profile of AYB Flour**

292 Steeping legumes in water or alkaline solution for varying 293 period is a traditional practice that impart positively on the 294 nutritional qualities of these legumes for those who consume 295 them. Amino acids are component subunits of proteins and are 296 used for growth and maintenance of living systems. The 297 observed higher levels of total amino acids and total essential amino acids in the flours from the steeped seeds relative to 298 299 unsteeped seeds prior to autoclaving (Table 1) could possibly be 300 due to the solubilization of some easy hydrolyzing components 301 and their migration to steeped solution resulting in amino acids increase by concentration effect [28, 29]. This result is in 302 agreement with the findings of Bujang and Taib [20] who 303 reported that soaking of soybean, groundnut and garbanzo 304 305 beans for 18h resulted in increases in amino acid contents of the 306 soaked product. Ferial and Esmat [30] also reported that soaking for 12hr increased the essential amino acids content in 307 308 chickpea seeds by 8.76% and attributed the increment to the 309 hydrolytic breakdown of the components during soaking. Other 310 authors [19, 29, 31] similarly reported of increases in protein 311 content of legume seeds during soaking.

312 The long-term steeping (48 - 72h) might have resulted in increased multiplication of microflora in the seeds and this might 313 314 have contributed to increase in protein content of the flour with 315 steeping period. Balogun [19] had similarly reported that the 316 protein and essential amino acids content of Bauhinra seed meal increased with soaking time ranging from 30.81% and 0.63% 317 for seeds that were soaked for 24h to 32.45% and 0.64% for 318 319 seeds that were soaked for 96h. The observed glutamic acid as 320 the major contributor in the total amino acids and leucine as the 321 major contributor in the total essential amino acid are in accordance with the report by Bujang and Taid [20] for soybean, 322 323 groundnut and garbanzo beans.

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326 4.2 Effect of Treatment on pH, Total Titratable Acidity and 327 Anti-nutrients in the AYB Flour

329 Steeping of dry cereal and legume seeds is usually 330 associated with migration of the steeped water or solution into the steeped seeds. Therefore, the observed highest pH value 331 332 (5.81) and lowest total titratable acidity value (0.10%) for the 333 flour from seeds that were soaked for 24h (Table 2) could be 334 attributed to the migration of the ash solution (alkaline solution) into the steeped seeds. The drop in pH and increase in total 335 336 titratable acidity with extension of steeping time to 48 and 72h 337 might be attributed to the actions of micro-organisms which could have induced acidity. The acid produced in the steeping 338 339 solution might have diffused into the seed thereby increasing the total titratable acidity content of the flour from the seeds 340 341 that were steeped for 48 and 72h. Hassan *et al.* [32] similarly 342 reported of decrease in pH and increase in total titratable acidity 343 of cocoyam soaked in distilled water for 72h. Mulyowidarso et al. 344 [33] reported that lactic acid bacteria dominate during the 345 soaking stage of a traditional process and as a result, a 346 significant increase in organic acids takes place.

African yam bean seed is a valuable source of protein for human consumption [7, 8]. However, phytate, tannin and trypsin inhibitor present in the seed as anti-nutrients hinder

350 digestion of protein and suppress release of amino acids for 351 absorption into the body thereby reducing nutritional value. 352 Result on Table 2 revealed that flour prepared from unsteeped but autoclaved seeds had significantly (p<0.05) higher levels of 353 354 phytate, tannin and trypsin inhibitors than the flour from 355 steeped and autoclaved seeds. The lower levels of these anti-356 nutrients in the flours from the steeped seeds relative to their 357 values in the flour from unsteeped but autoclaved seeds could 358 be attributed to their leaching out into the steeped solution 359 under the influence of concentration gradient. Steeping of 360 legumes in alkaline solution have been reported to enhance 361 permeability of the seed coat, soften the cotyledon and help the 362 leaching out of anti-nutrients into the steeping medium [34]. Other authors had similarly reported of reduction of anti-363 364 nutrients in legume seeds as a result of soaking in water or 365 bicarbonate solution [21, 34, 35, 36]. Longer steeping time resulted in higher reduction of phytate, tannin and trypsin 366 367 inhibitor in the flour ranging from 39.42, 32.15 and 44.18% 368 reduction for the flour from seeds that were steeped for 24h to 369 80.81, 86.08 and 98.72%, respectively for flour from seeds that 370 were steeped for 72h. Similar observations were reported by other authors [37, 38]. Reduction of these anti-nutrients is 371 essential to improve the nutritional quality of the flour and 372

effective utilization of the full potential of the flour as a source ofprotein.

375 The oligosaccharides (raffinose, stachvose and verbascose) present in legume seeds have been identified as 376 377 one of the major contributors of flatulence [31, 39]. Soaking of 378 raw legumes in water or bicarbonate solution is recommended to reduce oligosaccharides and their attendant physiological effects 379 of flatulence with legume consumption [40]. The result of the 380 present study showed that flour from unsteeped seeds had 381 382 significantly (p < 0.05) higher contents of raffinose and stachyose 383 than flours from the steeped seeds. Reddy et al. [41] reported 384 that the raffinose and stachyose are soluble in water and that soaking beans in water and discarding the soaked water will 385 386 remove most of these sugars from the beans. The lower levels 387 of raffinose and stachyose in the flour from the steeped seeds 388 relative to their values in the flour from the unsteeped seeds could therefore be due to their leaching out into the steeped 389 solution. Similar observation had been reported by other authors 390 [18, 21, 42]. The extent of losses of both raffinose and 391 392 stachyose was enhanced as the steeping time was increased 393 ranging from 67.44 and 68.63% reduction for the flour from the seeds that were soaked for 24h to 95.35 and 92.17%, 394 respectively for the flour from the seeds that were steeped for 395

396 72h. This observation is in accordance with the report by
397 Vijayakumari *et al.* [39].

399 **4.3 Effect of Treatment on Functional properties of AYB Flour**

Functional properties like bulk density, water absorption 400 401 capacity, oil absorption capacity, foaming capacity and swelling capacity of flour are critical in determining the suitability of such 402 403 flour for a given purpose. The effect of steeping time prior to 404 autoclaving on the functional properties of African yam bean 405 flour as presented on Table 3 revealed that steeping duration 406 had varying effects on the functional properties of the flour. The 407 insignificant (p>0.05) reduction in bulk density of the flours with increasing steeping time could be attributed to decrease in 408 409 carbohydrate with steeping time. According to Bhattachrya and 410 Parkash [43], bulk density of flour increased with starch 411 content. Similar reduction of bulk density as a result of soaking 412 had been reported by other authors [44,45]. Bulk density is one 413 of the parameters that help to decide the packaging material 414 [45]. The low bulk densities exhibited by the flours are desirable 415 in infant food production. The lower their values, the greater the 416 amount of flour particles that can bind together leading to 417 higher energy value [46].

418 All the flours exhibited high oil and water absorption 419 capacities (Table 3). The increase in oil absorption capacity with

420 longer steeping time could be attributed to increase in protein 421 content with steeping time. This observation is in agreement 422 with the reports by Agume et al. [35] and Desalegn [45]. The chemical component affecting oil absorption capacity is protein 423 424 which is composed of both hydrophilic and hydrophobic parts. 425 Non-polar amino acid side chain can form hydrophobic 426 interaction with hydrocarbon chain of lipid [47]. Oil absorption 427 capacity is of great importance since fat acts as flavour retainer, increases the mouth feel and improves palatability of food [48]. 428 429 The high oil absorption capacity of the flour samples suggests 430 that they could be useful in the preparation of bakery products, 431 sausages and doughnuts [46].

432 The result showed that both water absorption capacity and swelling index insignificantly (p>0.05) decreased with steeping 433 434 time (Table 3). This could be attributed to reduction in 435 carbohydrate with steeping time. According to Tester and 436 Morrison [49], the swelling capacity is due to the amount of 437 amylopectin fraction in starch, which is subject to degration during soaking. Yellavila et al. [50] noted that swelling power is 438 439 a measure of hydration capacity because the determination is a 440 weight measure of swollen starch granule and other occluded 441 water. Water absorption and swelling capacities are important 442 parameters which ultimately determine sample consistency and 443 are dependent on compositional structure of the sample [51].

444 Also, both water absorption and swelling capacities contribute to 445 dough formation and stability [52]. The water absorption 446 capacity and swelling index results obtained in this study are in 447 accordance with the report by Aguma et al. [35]. Both the flour 448 from unsteeped and steeped seeds exhibited very poor foaming 449 capacity. The insignificant (p < 0.05) increase in foaming capacity 450 of the flour with longer steeping time (Table 3) could be due to 451 increase in protein content with steeping time. According to 452 Yellavila et al. [50], foaming capacity generally depends on the 453 interfacial film formed by protein, which maintains the air 454 bubbles in suspension and slows down the rate of coalescence.

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456 **5.** CONCLUSION

457 The present study demonstrated that steeping African yam bean 458 seeds in 0.50% solution of unripe plantain peel ash for 24 to 72h prior 459 to autoclaving resulted in enhancement in total amino acids and total 460 essential amino acids. Conversely, phytate, tannin, trypsin inhibitor 461 and flatulence causing raffinose and stachyose significantly (p<0.05)462 decreased with steeping time. Bulk density, water absorption capacity 463 and swelling index decreased while oil absorption and foaming 464 capacities increased with steeping time.

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466 **Competing Interest**

467 No competing interest

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470		REFERENCES
471 472 473	1.	Igbal A, Kahlil IA, Ateeq N, Khan SM. Nutritional quality of important food legumes. Food Chem. 2006, 97(2):331-335.
474 475 476 477	2.	Rehman ZU, Shah WH. Domestic processing effect on some insoluble dietary fibre component of various food legumes. Food Chem. 2004, 87:613-617.
478 479 480 481 482	3.	Yin SW, Tang CH, Wen QB, Yang XQ, Li L. Functional properties and in vitro trypsin digestibility of red kidney bean (<i>Phaseolus</i> <i>vulgaris</i>) protein isolate effect of high pressure treatment. Food Chem. 2008, 110:938-945.
483 484 485 485	4.	Troszynska A, Estrella I, Lopez-Amores ML, Hermandez T. Antioxidant activity of pea (<i>Pisum sativum L.</i>) seed coat acetone extract. LWT Food Sci Technol. 2002, 35:158-164.
480 487 488 489 490	5.	Siddhuraju P, Manian S. The antioxidant activity and free radical-scavenging capacity of dietary phenolic extracts from horse gram (<i>Marcrotyloma unfilorum</i> (Lam) Verda) seed. Food Chem, 2007, 105:950-958.
491 492 493 494 495 496 497	6.	Doss A, Pugalenthi M, Vadivel VG, Subhashini G, Anitha-Subash R. Effect of processing technique on the nutritional composition and antinutrients content of uder-utilized food legume (<i>Canavalia ensiformis L. DC</i>). Inter. Food Res. J. 2011, 18(3):065-970.
498 499 500 501	7.	Apata DF, Ologhobo AD. Some aspects of biochemistry and nutritive value of African yam bean seeds (<i>Sphenostylis stenocarpa</i>). Food Chem. 1990, 36:271-280.
502 503 504 505	8.	Adewale B, Daniel A, Aremu CO. The nutritional potentials and possibilities in African yam bean for Africans. Inter. J. Agric. Sci. 2013, 3(1):8-19.
505 506 507	9.	Edem DO, Amugo CI, Eka OU. Chemical composition of yam bean (<i>Sphenostylis stenocarpa</i>). Trop. Sci. 1990, 30:59-63.
508 509 510 511	10.	Ajibade SR, Balogun MO, Afolabi OO, Ajomale KO, Fasoyiro SB. Genetic variation in nutritive and anti-nutritive content of African yam bean. Trop. Sci. 2005, 5:144-148.

512		
513	11.	Nwosu JN. Evaluation of the proximate composition and anti-
514		nutritional properties of African vam bean (Sphenostylis
515		stenocarpa) using malting treatment. Inter. 1. Basic Appl. Sci.
516		$2013 2(4) \cdot 157 - 169$
517		2013, 2(4):137 103.
518	17	Ajibola CO. Olanade AA. Physical provimate and anti-nutritional
510	12.	Ajibola GO, Olapade AA. Friysical, proximate and anti-nutritional
519		composition of African yain bean (<i>Sphenostylis Steriocarpa</i>) seed
520		varieues. J. Food Res. 2016, $5(2):67-72$.
521		
522	13.	Shimelis EA, Rakshit SK. Effect of processing on anti-nutrients
523		and in vitro digestibility of kidney bean (<i>Phaseolus vulgaris L.</i>)
524		varieties grown in East Africa. Food Chem. 2007, 103:161-172.
525		
526	14.	Ene-Obong HN, Obizoba IC. Effect of domestic processing on the
527		cooking time, nutrients, anti-nutrients and in vitro protein
528		digestibility of African vam beans (Sphenostylis stenocarpa).
529		Plant Foods Hum Nutr 1996 49.43-52
530		
530	15	Ildensi EA Arisa NII Ikna E Effect of soaking and boiling and
533	15.	anutoclaving on the nutritional quality of Mucuna flagollings
522		(uluna) Afr. 1 Discham Des 2010 4(2):47 E0
533		(ukpo). All. J. Biochem. Res. 2010, $4(2)$.47-50.
534	1.0	
535	16.	D'souza MR. Effect of traditional processing method on
536		nutritional quality of field bean. Adv. Biores. 2013, 14(3):29-33.
537		
538	1/.	Mubarak AE. Nutritional composition and antinutritional factors
539		of mung bean seed (<i>Phaseolus aureus</i>) as effected by some
540		home traditional processes. J. Food Chem. 2005, 89(4):489-
541		495.
542		
543	18.	Silva HC, Braga GL. Effect of soaking and cooking on the
544		oligosaccharides content of dry bean (<i>Phaseolus vulgaris L.</i>). J.
545		Food Sci. 2006, 47(3):924-925.
546		
547	19.	Balogun BI. Effect of processing on the proximate composition
548		and amino acid profile of <i>Bauhinia monandra</i> (Kurz) seed.
549		Academic 1 Interdis Studies 2013 2(12):144-151
550		
551	20	Buiang A Taib NA Changes on amino acid content in sovhean
552	20.	arbanzo bean and groundnut during pro-treatments and tempo
		making Saine Malaysiana 2014 42(4) EE1 EE7
222		Making. Sams Malaysiana. 2014, 45(4).551-557.
554	24	
555	∠⊥.	EI-Adawy IA. Nutritional composition and anti-nutritional factors
556		or chickpeas (<i>Cicar arietinum L.</i>) undergoing different cooking
55/		methods and germination. Plant Foods Hum. Nutr. 2002, 57:83-
558		97.

559 560 22. Spackman DH, Stein EH, Moore S, Automatic recording 561 apparatus for use in the chromatography of amino acids. Analy. 562 Chem. 1958, 30:1191-1197. 563 AOAC. Official methods of analysis (17th edn.) Association of 564 23. 565 Official Analytical Chemists, Washington DC, USA. 2005. 566 567 24. Oberleas D. Phytate. In: Toxicants occurring naturally in foods. 568 F. Strong (ed.). National Academy of Science, Washington DC. 569 1973, pp. 363-371. 570 571 25. Arntfield SD, Ismond MAH, Murray ED. The fate of anti-572 nutritional factors during the preparation of faba bean protein 573 isolate using micellization technique. Can. Inst. Food Sci. J. 574 1985, 18:137-143. 575 576 26. Okezie BO, Bello AE. Physico-chemical and functional properties 577 of winged bean flour and isolate compared with soy isolate. J. 578 Food Sci. 1988, 53(2):450-455. 579 580 27. Abbey BN, Ibeh GO. Functional properties of raw and heat 581 processed cowpea (Vigna unquiculata) flour. J. Food Sci. 1988, 582 53(6):1775-1778. 583 584 Filipiak-Florkiewiez A, Florkiewiez A, Cieslik E, Walkowska I, 28. 585 Walezyeka M, Leszezynska T, Kapusta-Duch J. Effect of various 586 hydrothermal treatments on selected nutrients in legume seeds. 587 Polish J. Food Nutr. Sci. 2011, 6(3):181-186. 588 589 29. Winiarska-Mieczan A, Koczmara K. Effect of soaking on the 590 nutritional value of kidney bean (Phaseolus vulgaris), soybean 591 (Glycine max) and lentil (Lens culinaris) seeds. Acta Agro-592 physica. 2006, 8:537-543. 593 594 Ferial MAS, Esmat AAA. Physico-chemical properties of tempe 30. 595 produced from chickpea seeds. J. Amer. Sci. 2011, 7(7):107-596 118. 597 598 31. Ramadam EA. Effect of processing and cooking methods on the 599 chemical composition, sugars and phytic acid of soybean. Food 600 Publ. Health. 2012, 2(1):11-15. 601 602 Hassan GF, Yusuf L, Adebolu TT, Onifade AK. Effect of 32. 603 fermentation on mineral and anti-nutritional composition of 604 cocoyam (Colocasia esculenta Linn.). Sky J. Food Sci. 2015, 605 4(4):042-049.

606 607 33	Mulyowidarso RK Elect GH Buskle KA Changes in the
608 609 610	concentration of organic acids during soaking of soybeans for tempe production. Inter. J. Food Sci. Technol. 1991, 26(5):607- 614.
612 34. 613 614 615 616	Sharma S, Goyal R, Barwal S. Domestic processing effect on physico-chemical, nutritional and anti-nutritional attributes of soybean (<i>Glycine max L. Merill</i>). Inter. Food Res. J. 2013, 20(6):3203-3209.
617 35. 618 619 620	Agume ASN, Njintang NY, Mbofung CM. Effect of soaking and roasting on the physico chemical and pasting properties of soybean flour. Foods. 2017, 6, 12; doi:10.3390/foods6020012.
621 36. 622 623 624 625	Mugabo F, Afoakwa EO, Annor G, Rwubatse B. Effect of pretreatment and processing conditions on anti-nutritional factors in climbing bean flour. Inter. J. Food Studies. 2017, 6:34-43.
626 37. 627 628 629 630	Rasha MK, Esmat AA, Gibriel AY, Nagwa MH, Ferial MA. Effect of legume processing treatment individually or in combination on their phytic acid content. Afr. J. Food Sci. Technol. 2011, 2(2):036-046.
631 38. 632 633 634 635	Adeleke OR, Adiomo OO, Fawale OS, Olamiti G. Effect of soaking and boiling on anti-nutritional factors, oligosaccharide content and protein digestibility of newly developed bambara groundnut cultivars. Food Sci. Technol. 2017, 5(9):1006-1014.
636 39. 637 638 639 640	Vijayakumari K, Pugalenthi M, Vadivel V. Effect of soaking and hydrothermal processing method on the levels of anti-nutrients and in vitro protein digestibility of <i>Bauhinia purpurea</i> L. Seed. Food Chem. 2007, 103:968-975.
641 40. 642 643 644 645	Niba LL, Rose N. Effect of soaking solution concentration on resistant starch and oligosaccharide content of adzuki (<i>V. angularis</i>), Fava (<i>F. faba</i>), Lima (<i>P. lunatus</i>) and mung bean (<i>V. radiate L.</i>). J. Food Sci. 2003, 1(1):4-8.
646 41. 647 648	Reddy NR, Sathe SK, Salunkhe DK. Phytates in legumes and cerials. Adv. Food Res. 1982, 28:1-92.
649 42. 650 651 652	Taiwo KA, Akanbi CT, Ajibola OO. Regression relationship for the soaking and cooking properties of two cowpea varieties. J. Food Engr. 1998, 37:331-344.

660

664

667

671

679

- 653 43. Bhattachrya S, Prakash M. Extrusion blends of rice and chicken
 654 pea flour: a response surface analysis. J. Food Engr. 1994,
 655 21:315-330.
- 657 44. Ocheme OB, Chinma CE. Effect of soaking and germination on
 658 some physico-chemical properties of millet flour for porridge
 659 production. J. Food Technol. 2008, 6(5):185-188.
- 661 45. Desalegn BB. Effect of soaking and germination on proximate
 662 composition, mineral bioavailability and functional properties of
 663 chickpea flour. Food Publ. Health. 2015, 5(4):108-113.
- 665 46. Onimawo AI, Egbekun KM. Comprehensive Food Science and 666 Nutrition. Macmillan Publishers, Ibadan. 1998, p. 228.
- 47. Jitngarmkusol S, Hongsuwankus J, Tananuwong K. Chemical
 composition, functional properties and microstructure of
 defatted macademice flours. Food Chem. 2008, 110:23-30.
- 48. Amandikwa C, Iwe MO, Uzomah A, Olawuni AI. Physico-chemical
 properties of wheat-yam flour composite bread. Nig. Food J.
 2015, 33(1):12-17.
- 676 49. Tester. RF, Morrison WR. Swelling and gelatinization of cereal
 677 starches. I. Effect of amylopectin, amylase and lipids. J. Cereal
 678 Chem. 1990, 67:551-557.
- 680 50. Yellavila SB, Agbenorhevi JK, Asibuo JY, Sampson GO. 681 Proximate composition, mineral content and functional 682 properties of five lima bean accessions. J. Food Security. 2015, 683 3(3):69-74. 684
- 685 51. Ayo-Omogie A, Ogunsakin R. Assessment of chemical,
 686 rheological and sensory properties of fermented maize-cardaba
 687 banana complementary food. Food Nutr.Sci. 2013, 4(8):844688 850.
- 690 52. Olapade AA, Oluwole OB. Bread making potential of composite
 691 flour of wheat-acha (*Digitariae exilis staph*) enriched with
 692 cowpea (*Vigna uncuiculata L. Walp*) flour. Nig. Food J. 2013,
 693 31(1):6-12.

694 695