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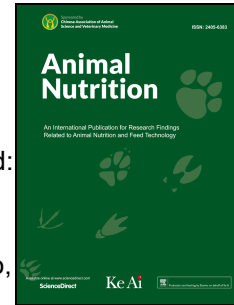
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1 **Processing techniques of selected oilseed by-products of potential use in animal feed: Effects**
2 **on proximate nutrient composition, amino acid profile and antinutrients**

3

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19 **ABSTRACT**

20 The effects of processing by autoclaving (AC), soaking (SK), short-term fermentation (S-TF, 4 d)
21 and long-term fermentation (L-TF, 14 d) on the nutritional composition, amino acid profile and
22 some antinutrients were determined for cottonseed meal (CSM), groundnut meal (GNM) and
23 groundnut husk (GH) in this study. After processing, crude protein content improved by 11% after
24 L-TF, and crude lipid content 25% after SK for CSM; crude protein content improved by 27% after
25 S-TF and L-TF, and crude lipid content 13% after SK for GNM. Soaking and fermentation were
26 shown to significantly increase essential amino acid contents by 44% (SK, methionine) in CSM and
27 46% in GNM (L-TF, histidine). Phosphorus content was reduced by 59% in CSM and 57% in GNM
28 by L-TF. All processing techniques, with the exception of AC, reduced phytic acid and gossypol
29 contents in CSM and GNM. It was concluded that SK and fermentation were simple, cost-effective,
30 and efficient ways to improve the nutritional value of the selected oilseed by-products.

31 **Keywords:** Amino acid; Autoclaving; Fermentation; Proximate composition; Soaking

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33

34 1. Introduction

35 As the production volume of fish meal has leveled off in recent years, the commodity price has
36 risen, driving research to focus on more sustainable, non-marine alternatives of dietary protein
37 sources (Schipp, 2008; Cocker, 2014) to satisfy rising demands from the animal production sector.
38 Most often, agro-industrial by-products that are used in animal feeds are of modest economic value,
39 but of reliable quantity (Agbo, 2008). Many plant-based feed resources that could be of
40 considerable nutritional and financial value in animal production remain unexploited, undeveloped
41 or poorly utilized (Agbo and Prah, 2014). Under-utilization and disposal of these resources are
42 likely due to a lack of adequate information on how their nutritional quality could be improved.
43 Considering the expected increase in world population and the high demand for animal products
44 due to growth in most world economies, the prospect of feeding millions and safeguarding their
45 food security will depend on the better utilization of non-conventional feed resources and
46 implementation of circular bio-economy (NoRest, 2016).

47 Agro-industrial by-products, especially residual oilseed cakes and meals from oil extraction, are
48 available in large quantities. Global production reached 317,000,000 t in 2016 and is forecasted to
49 rise to 386,000,000 t by 2025 (OECD/FAO, 2016). Most of these protein meals have been explored
50 as feed ingredients in their unrefined state to replace fish meal as alternative protein sources,
51 especially for poultry, pigs and aquatic animals. Previous studies on oilseed meal-based diets fed to
52 various animals have reported negative, although highly variable, effects on production
53 performance. For instance, studies that shea nut meal based diets were fed to broiler chickens
54 (Atuahene et al., 1998) and Nile tilapia (Agbo et al., 2014), observed low growth performance
55 caused by poor digestibility, and possibly reduced feed intake (Elemo et al., 2011). Dabrowski and
56 Kozak (1979) observed a lower growth performance in grass carp fry fed with different levels of
57 commercial soybean meal compared to fishmeal. Weaning pigs fed increasing levels (5% to 15%)

58 of copra and palm kernel expeller meals showed a linear reduction in final body weight, while no
59 difference in growth performance was recorded with palm kernel meal compared to a control diet
60 containing soybean meal and 4% of fish meal (Jaworski et al., 2014).

61 The usefulness of these by-products are either partly caused by, or further restricted by, the
62 presence of antinutrients such as trypsin (protease) inhibitors, tannins and lectins, phytate,
63 gossypol, oxalates and glucosinolates, saponins, antivitamins, and mycotoxins (Francis et al., 2001).
64 These compounds affect protein and mineral utilization (Francis et al., 2001; Pashwar, 2005) by
65 decreasing palatability, digestibility, or metabolism, and may even exert a toxic effect resulting in
66 liver damage (Pashwar, 2005).

67 There is a need to increase the nutritional value of oilseed by-products, and to offset certain
68 antinutrients and toxins, in order to realize their full potential as animal feed ingredients (Annongu
69 et al., 1996; Pashwar, 2005). Techniques such as fermentation (Lopez et al., 2001), boiling and
70 sodium hydroxide (NaOH) treatment (Annongu et al., 1996), heating and/or autoclaving (AC)
71 (Clatterbuck et al., 1980), and sprouting or germination (Asiedu et al., 1993) have been proposed as
72 ways of detoxifying and improving the nutritional value of these feed ingredients. The current study
73 was designed to assess the effect of processing cottonseed meal (CSM), groundnut meal (GNM)
74 and groundnut husk (GH) by AC, soaking (SK), short-term fermentation (S-TF) or long-term
75 fermentation (L-TF) on the proximate composition, amino acid profile and some antinutrients .
76

77 **2. Materials and methods**

78 **2.1. Sources and preparation of raw materials**

79 Groundnut husk was purchased from a groundnut paste processing factory, mechanically
80 extracted GNM from a local producer, and screw-pressed CSM was purchased from a commercial
81 agro-feed seller, all in Kumasi, Ghana. Prior to powdering with a hammer mill, the GNM was dried

82 in an oven (Gallenkamp Hotbox Oven) at 100 °C for 24 h, and cooled in a desiccator at room
83 temperature. The other ingredients were also finely ground using a commercial hammer mill. All
84 ingredients were then sealed in airtight bags and shipped to the Technical University of Denmark
85 (DTU Aqua) where they were kept at -20 °C until needed for further processing. Commercial grade
86 dried baker's yeast (*Saccharomyces cerevisiae*) used in the fermentation process was purchased
87 from a local supplier in Denmark.

88 **2.2. Processing procedures**

89 The processes of AC, SK, S-TF and L-TF were performed on 100 g samples of CSM, GNM and
90 GH weighed out on an electronic scale (Mettler Toledo, XS4002S, Switzerland) in triplicate.
91 Samples of each raw material were treated as unprocessed (UP).

92 *2.2.1. Autoclaving*

93 The samples of CSM, GNM, and GH were transferred to 500 mL Duran glass bottles. Distilled
94 water was added at a ratio of 7:3 (w/V) and mixed thoroughly before AC at 120 °C for 20 min. The
95 samples were then allowed to cool to room temperature (20 °C), after which they were oven dried
96 (Memmert, UN110) at 40 °C until constant weight, cooled and stored at -20 °C until analysis.

98 *2.2.2. Soaking*

99 The samples of CSM, GNM and GH were transferred to 2 L glass jars. Tap water was added at a
100 ratio of 1:10 (w/V). The samples were allowed to soak at room temperature for 12 h with
101 intermittent stirring every 4 h after which the water was decanted. The samples were transferred
102 onto a fine meshed cloth (100 µm) and squeezed, to remove as much of the water as possible. The
103 residual meal was spread on a tray and oven dried (Memmert, UN110) at 40 °C to constant weight.
104 After drying and cooling to room temperature, the samples were finely ground, sealed in polythene
105 bags, and stored at -20°C until analysis.

106 2.2.3. Fermentation

107 For S-TF and L-TF, CSM, GNM and GH was transferred to 500 mL Duran glass bottles, and
108 inoculated with 3.40 mg of dried baker's yeast (*Saccharomyces cerevisiae*). Tap water (80 mL) was
109 added and mixed thoroughly before fermenting for either 4 or 14 d at room temperature in a sealed
110 bottle. At the end of the fermentation process, the samples were soaked in 300 mL of tap water at
111 room temperature for 5 min. Water removal, drying and storage followed the procedure described in
112 section 2.2.2.

113 2.3. Analytical procedure for proximate composition, amino acid profile and antinutrients

114 Dry matter, crude protein and ash contents of the unprocessed and processed samples were
115 determined following the procedures of the Association of Official Analytical Chemists (2005). Dry
116 matter content was determined after oven drying for 24 h at 105 °C (Mettler UN110). Ash
117 content was determined by incineration of the samples for 6 h at 550 °C in a muffle furnace
118 (Heraeus Instruments K1252). Crude protein content was determined by the Kjeldahl method
119 (FOSS Kjeltex 2200) and crude lipid content by the method described by Bligh and Dyer (1959).
120 Phosphorus content was determined in accordance with ISO 6491:1998 (1998) standard method.
121 The amino acid profile of the experimental ingredients were determined in duplicates by High
122 Performance Liquid Chromatography (HPLC) analyses following the method of Larsen et al.
123 (2011). Gossypol content analyses followed the procedure described by Pons and Hoffpauir (1954).
124 Phytic acid content was determined using a commercially available kit (K-PHYT, Megazyme,
125 Ireland) based on the method described by Fiske and Subarrow (1925).

126

127 2.4. Experimental design and Statistical analysis

128 The oilseed by-products namely CSM, GNM and GH were subjected to 4 treatment processes by
129 AC, SK, S-TF, and L-TF in addition to unprocessed samples. Each treatment was replicated 3 times

130 per by-product and analysed in duplicates which gave the total number of observation as 3 (oilseed
131 by-products) \times 5 (treatments) \times 3 (replicates) = 45 for each variable. The Shapiro-Wilk normality
132 test was performed on data for each variable before that the averages of the processed samples were
133 subjected to a one-way ANOVA at $P < 0.05$. The differences between the means of the unprocessed
134 and the processed raw materials were determined by the Dunnett's multiple comparison test using
135 GraphPad Prism 5.01 statistical software for Windows (San Diego California, USA). Results of the
136 effect of AC, SK and fermentation against the raw materials are expressed as means with their
137 standard deviations (SD), and percentage changes in variables are presented in figures.

138

139 **3. Results**

140 *3.1. Proximate composition*

141 After 12 h of SK, S-TF (4 d) and L-TF (14 d), respectively, the nutritional contents of CSM,
142 GNM and GH were significantly ($P < 0.05$) affected (Table 1). Autoclaving did not have any major
143 effect on nutritional composition of the raw materials tested. Dry matter (DM) content of CSM
144 appreciably increased by 5.50% ($P < 0.0001$) after 14 d of fermentation. In CSM, crude protein
145 content was the highest (463.45 g/kg DM) after L-TF and the lowest (447.15 g/kg) after SK except
146 AC. Autoclaving however, resulted in approximately 9% reduction in crude protein content of
147 CSM. Improvement in crude lipid content ranked as follows: SK $>$ L-TF $>$ S-TF. Meanwhile, ash
148 and phosphorus contents were drastically reduced by approximately 52% and 59%, respectively,
149 after L-TF. In GNM, dry matter content was reduced by AC (1.72% reduction), whereas S-TF and
150 L-TF increased dry matter content by about 3%. After processing, crude protein content varied
151 widely in GNM ranging from 416.40 g/kg DM after AC to 528.75 g/kg DM after S-TF, which
152 corresponded to increments between 0 and 27%, respectively. Crude lipid content was increased by
153 13%, 5%, and 12% after SK, S-TF, and L-TF, respectively. Ash and phosphorus contents were

154 reduced ($P < 0.0001$) by all treatment processes except AC. Phosphorus reduction was the lowest
155 (59%) after L-TF and the highest (3%) after AC. At the end of the treatment processes on GH,
156 marginal increases were recorded; crude protein and crude lipid contents were increased by 3% and
157 11%, respectively, after SK. Ash content was considerably reduced by up to approximately 22%
158 after SK. Phosphorus content was reduced between 23% and 30% by SK, S-TF and L-TF ($P =$
159 0.0002).

160 3.2. Amino acid profile

161 The amino acid profile for the unprocessed and processed by-products are presented in Tables 2
162 to Table 4. Apart from AC, the other processing techniques (SK, S-TF and L-TF) induced
163 significant changes ($P < 0.05$) especially in the essential amino acids (EAA) profile of the selected
164 oilseed meals. Total amino acids (TAA) as a percentage of the calculated crude protein ranged from
165 74% to 86%.

166 3.2.1. Cottonseed meal

167 Autoclaving CSM improved all EAA except lysine and methionine (Fig. 1A), and resulted in an
168 overall increase in total essential amino acids (TEAA) of 11% (Table 2). All of the EAA and non-
169 essential amino acids (NEAA) increased after SK, S-TF and L-TF. For the EAA, the highest
170 increment was recorded for methionine after L-TF at 44%, while the lowest increment was
171 observed for isoleucine (5%) also after L-TF. Soaking and S-TF processes increased TEAA by 31%
172 and 28%, respectively, of which the majority came from methionine, and the minority from lysine
173 (Fig. 1B to Fig. 1D). Total non-essential amino acids (TNEAA) content was unaffected by AC, but
174 increased by 16% to 18% after SK, S-TF, and L-TF (Table 2).

175 3.2.2. Groundnut meal

176 The majority of the EAA and NEAA in GNM at the end of the study increased after SK, S-TF
177 and L-TF ($P < 0.05$) (Table 3), whereas the effects from AC treatment were marginal with a
178 tendency to decrease (Fig. 2A). Increments in EAA in GNM varied widely from 8% in lysine to
179 26% in phenylalanine after SK; from 18% and 12% in lysine to 40% and 46% in histidine after S-
180 TF and L-TF, respectively (Fig. 2B to Fig.2D). All other EAA were increased by about one-quarter
181 after SK and about one-third for both S-TF and L-TF. Among NEAA, glutamic acid was essentially
182 unaffected whereas hydroxyproline increased by 23% after SK. The S-TF and L-TF processes
183 notably increased the NEAA content; for aspartic acid by 4% and for alanine by 61%. Overall, the
184 TEAA accounted for 43% to 45% of the measured crude protein after processing, compared to 43%
185 in the unprocessed sample (Table 3).

186 3.2.3. Groundnut husk

187 Groundnut husk had a very low amino acid contents in the unprocessed form ranging from 0.13%
188 to 1.39% for the EAA, with methionine being the least and arginine the most abundant (Table 4).
189 Fermentation and SK of GH appears to lead to an inhibition of the derivatization of amino acids by
190 the 6-aminoquinolyl-N-hydrosysuccinimidyl carbamate (AQC) used, therefore amino acids analysis
191 in these treatments was not possible. Autoclaving of GH reduced lysine, methionine, alanine,
192 aspartic acid, and glutamic acid contents by 25%, 38%, 16%, 31%, and 26% ($P < 0.05$),
193 respectively (Fig. 3), whereas cysteine content was improved (31%) ($P < 0.05$).

194

195 3.3. Nitrogen constituents

196 The total nitrogen (TN), total amino acid nitrogen (TAA-N) and non-protein nitrogen (NPN)
197 before and after processing are given in Table 5. Overall, TN increased slightly in GNM, but was
198 marginally reduced in CSM and GH after AC. Nonetheless, considerable TN gains (up to 27% in

199 GNM) were recorded in all SK and fermented samples. Similarly, NPN doubled after SK in GNM,
200 and reduced by half after AC in CSM. On the other hand, TAA-N increased by approximately 25%
201 after SK, S-TF and L-TF in CSM, and between 14% and 26% after SK and S-TF in GNM,
202 respectively.

203 3.4. Anti-nutritional factors

204 The results of the effect of processing on the gossypol and phytic acid contents in CSM, GNM
205 and GH are presented in Table 6. Autoclaving of CSM resulted in the largest degradation of
206 gossypol, removing 34% ($P = 0.0043$), followed by SK and fermentation. Short-term fermentation
207 was the most efficient means of removing gossypol in GNM (45%, $P = 0.0041$) and GH (67%, $P =$
208 0.0005). Long-term fermentation was found to be most efficient in decreasing phytic acid from both
209 CSM (72%, $P < 0.0001$) and GNM (69%, $P = 0.0003$), whereas the lowest degradation was
210 recorded after AC.

211 4. Discussion

212 4.1. Proximate composition

213 The moderate losses in crude protein content from AC raw materials were not significant in
214 comparison to unprocessed samples, and do not appear critical. Nonetheless, these losses could be
215 nutritionally detrimental if specific amino acids were more sensitive to AC treatment than others.
216 The extent of protein change or destruction has been correlated with duration and temperature of
217 AC treatment, as well as moisture content (Goh et al., 1979; McNaughton and Reece, 1980;
218 Papadopoulos, 1989). This effect was demonstrated by Chrenkova et al. (1986) who found that
219 lengthy exposure time (60 to 130 min) coupled with high hydrothermic temperatures (110 to 130
220 °C) significantly decreased soluble crude protein content in soybean meal, alfalfa meal, wheat meal
221 and field pea. Although the samples in the present study were autoclaved at high temperature (121
222 °C), the relatively short time of exposure (20 min) could account for the moderate losses observed.

223 Nonetheless, these losses are not regarded as critical especially as the nitrogen contents in the
224 samples were not limited.

225 Soaking and fermentation (S-TF and L-TF) positively affected the crude protein and crude lipid
226 contents of the CSM and GNM tested. These are comparable to the report of Mukhopadhyay and
227 Ray (1999), in which marginal increases in protein (3.28%) and lipid (17.54%) contents of sesame
228 seed meal were found after combined SK and fermenting with lactic acid bacteria (*Lactobacillus*
229 *acidophilus*). They indicated that although, small nutrient losses occur during fermentation and SK
230 through microbial utilization or leaching, while increases occur through microbial synthesis.
231 Similarly, Sun et al. (2015) reported a net protein increment of 7.6% in CSM after fermentation
232 with *Bacillus subtilis*. In the current study, fermentation increased crude protein contents in the
233 fermented oilseed meals and by-product between 1% in GH to 27% in GNM. The higher protein
234 levels in this study were likely due to longer duration of fermentation which allowed the yeast to
235 convert NPN into amino acids. Single cell proteins (SCP) such as yeast, contain 45% to 65% crude
236 protein, and 2% to 6% crude lipid on a dry weight basis (Nasseri et al., 2011). In all likelihood, the
237 increased protein content after fermentation resulted from yeast cells mixed with the fermented
238 samples at termination of experiment. After 12 h of SK mungbean, Sattar et al. (1989) reported
239 approximately 5% and 9% increases in protein content, with a positive temperature correlation. The
240 increase in protein after SK in their work is somewhat similar to our observations for CSM (6.71%),
241 while our results for GNM were considerably higher (22.30%). These positive changes in protein
242 content in the oilseed by-products may be attributed to the breakdown of soluble starch and losses
243 of fine solids, which increased the relative contribution from protein.

244 The increased content of crude lipid after SK of oilseed meals in the present study contradicts
245 previous reports (Siddhuraju et al., 2000; Nwaoguikpe et al., 2011). However, the lipid increment
246 observed in this study could be the result of the leaching of soluble components that caused that the

247 content of lipid in the oilseed meals (Agume et al., 2017), and the destruction of cell structure
248 causing the efficient release of oil reserve (Cuevas-Rodriguez, 2004), which were probably retained
249 in the meals by the fine mesh cloth during removal of excess water.

250 Fermentation has only previously been shown to moderately alter ash content (Plaipetch and
251 Yakupitiyage, 2012; Sun et al., 2015). In the current study fermentation resulted in large reductions
252 in ash content, corresponding to 52% in CSM, 61% in GNM and 18% in GH. The loss in ash was
253 accompanied by decreases in phosphorus content for all samples. This could be due to the
254 hydrolysis of phytate by endogenous phytases which might have possibly transformed the free
255 phosphorus as a result of phytate degradation into other phosphorus compounds such as inorganic
256 phosphoric acids, orthophosphates and lower inositol phosphates (Türk et al., 2000; Shunmugam et
257 al., 2015). The reductive effect of SK on ash in all samples is likely due to the solubilisation of
258 some vitamins and minerals like phosphorus in the SK media (water) (Agume et al., 2017). In
259 general, some reductions could also be consequences of changes in other constituents such as
260 increases in crude protein and lipid contents.

261 4.2. Amino acid composition

262 Soaking, S-TF and L-TF improved the amino acid profile of CSM and GNM compared to
263 unprocessed samples. Generally, water-soluble amino acids are expected to be lost through SK yet,
264 the non-deleterious effect of SK on the amino acid profile could be linked to the plant by-products'
265 composition of higher proportions of insoluble amino acids that may primarily function as structural
266 parts of the plant (Wade, 2009). A possible explanation for the significant increases in most of the
267 amino acids observed for the fermented and soaked samples could be that the oilseed by-products in
268 their raw state contained sufficient quantities of NPN to meet the yeast's nitrogen requirements.
269 Alternatively, the amino acid contents of the raw samples were protein bound, and not available for
270 the yeast to assimilate (Vinquiry, 2014; Howell, 2011). Therefore, any amino acid synthesized by

271 the yeast ended up as an add-on contributing to the increase contents of amino acids in the
272 processed samples.

273 The heat treatment (AC) applied in this study had little incremental effect on the TAA content of
274 CSM, whereas no changes were recorded in GNM. However, major depletion occurred especially in
275 lysine and methionine in GH. These losses could be nutritionally detrimental since these EAA
276 cannot be synthesized by fed animals. This is in support of Papadopoulos (1989) and Bellagamba et
277 al. (2015) who concluded that heat processing of feedstuff causes the racemization of amino acids
278 and the formation of cross-linkages with resultant reduction in amino acid digestibility. Certain
279 amino acids like cystine and lysine are reported to be heat-sensitive even during limited exposure.
280 Many authors have also reported significant reductions in lysine, serine, arginine, and threonine
281 among others in CSM (Craig and Broderick, 1981), soybean meal (McNaughton and Reece, 1980)
282 and rapeseed meal (Goh et al., 1979) by AC. Fermentation, on the other hand, has been shown to
283 increase glutamic and aspartic acid contents in cocoa bean, groundnut, garbanzo bean and soybean
284 (Adeyeye et al., 2010; Bujang and Taib, 2014). These authors further reported significant increases
285 in lysine, histidine, arginine, serine, glycine, alanine, valine, isoleucine, tyrosine and phenylalanine
286 with a conclusion that fermentation particularly improves the EAA content of oilseed by-products.
287 Comparatively, similar increments were obtained in the current study. Furthermore, Bujang and
288 Taib (2014) recorded 71%, 63% and 53% enhancements in total amino acids in groundnut,
289 garbanzo bean and soybean, respectively, after 24 h of fermenting with *Rhizopus oligosporus*.
290 Transamination, has been proposed as a responsible mechanism for these increases (Baumann and
291 Bisping, 1995). In the current study, SK the oilseed by-products in water for 24 h resulted in
292 appreciable improvements in the majority of EAA. This agrees with Adeyeye (2008), Abu-Salem
293 and Abou-Arab (2011), and Bujang and Taib (2014) who recorded higher EAA contents in
294 *Sorghum bicolor* grains, chickpea seeds and groundnut, garbanzo bean and soybean, respectively.

295 Based on the present data on amino acids, SK and fermentation are very important processing
296 techniques that could be adopted by feed manufacturers to improve the nutritional quality of oilseed
297 by-products intended for use in animal feed at low cost.

298

299 4.3. Changes in nitrogen constituents

300 From a nutritional perspective, the increase in TN especially after SK and fermentation in GNM
301 is positive since it directly reflects an increase in crude protein. However, the elevated contents of
302 NPN compared to the TAA-N constituents is not ideal, since most fish possess simple stomachs that
303 lacks the mechanisms to effectively utilize NPN. The increase in NPN content is reported to be
304 partially influenced by the types of microorganism and endogenous proteolytic activity present
305 during fermentation (Demasi et al., 1990).

306 4.4. Anti-nutritional factors

307 The application of heat is widely accepted as a superior way of removing antinutrients that
308 affect nutrient digestibility. Autoclaving has proven efficient in reducing gossypol contents,
309 especially in CSM, but cooking has also been shown to be an efficient approach (Nagalakshmi et
310 al., 2002). Gossypol reduction in CSM by yeast fermentation in the current study was
311 approximately 17% for S-TF. This reduction for the fermented CSM is less than reported by Sun et
312 al. (2015) after solid state fermentation. Zhang et al. (2007) fermented CSM with 3 different yeast
313 strains (*Candida capsuligena*, *Candida tropicalis*, and *Saccharomyces cerevisiae*) and 3 different
314 fungi strains (*Aspergillus terricola*, *Aspergillus oryzae*, and *Aspergillus niger*) for 48 h. Their
315 results showed a reduction of free gossypol as high as 94.6% when CSM was fermented with
316 *Candida tropicalis* followed by *Saccharomyces cerevisiae* (88.5%), *Aspergillus niger* (85.2%) and
317 *Aspergillus terricola* (82.9%). These reductions in free gossypol were attributed to microbial or

318 enzymatic degradation of gossypol, or possibly the incorporation of free gossypol into gossypol-
319 protein complexes or gossypol-lipid complexes.

320 The content of phytic acid after 20 min of AC the oilseed by-products at a temperature of 121 °C
321 were reduced the most in GNM (approximately 15%). Similar observations were reported by
322 Embaby (2010), who recorded degradation in peanut seeds by 9.5% and 24.7% after AC at 121 °C
323 for 10 and 20 min, respectively. Agbo (2008) only reported marginal decreases in PA after AC at
324 121 °C for 30 min. Studies investigating SK times and temperatures show that phytate reduction is
325 somewhat dependent on pH and highly dependent on temperature (Sattar et al., 1989; Gustafsson
326 and Sandberg, 1995). This assertion is also supported by Lopez et al. (2001) who reported that in
327 addition to pH and temperature, water and duration are of importance. Furthermore, Abou-Arab and
328 Abu-Salem (2010) reported a 29% phytic acid reduction in whole seeds of *Jatropha curcas* after SK
329 in water for 12 h at room temperature, which is in line with results from the present study where
330 phytic acid was reduced by 40%, 39% and 31% for CSM, GNM and GH, respectively, after SK for
331 12 h at room temperature. Fermentation has been reported by many authors to reduce phytic acid
332 content in plant products irrespective of the type of fermenting agent used. The significant reduction
333 of phytic acid as a result of yeast fermentation in this study showed reductions to tolerable levels.
334 However, the differences in the extent of degradation (S-TF and L-TF) can be associated with the
335 length of time for fermentation. Likewise, significant depletion (52% to 70%) of phytic acid in
336 *Jatropha curcas* kernel cake was reported by Belewu and Sam (2010) after a 7 d solid-state
337 fermentation with 5 different types of fungi (*Aspergillus niger*, *Penicillium chrysogenum*, *Rhizopus*
338 *oligosporus*, *Rhizopus nigricans* and *Trichoderma longibrachitum*). Phytic acid content in yeast
339 fermented canola meal was reduced by approximately 8% after 24 h of fermentation according to
340 Plaipetch and Yakupitiyage (2012). According to a study by Fardiaz and Markakis (1981), phytic
341 acid reduction of up to 96% was recorded after fermenting peanut press cake for 72 h at 30 °C and

342 they attributed the decrease to the release of phytase by the moulds (*Neurospora sitophila* ATCC
343 14151, *Rhizopus oligosporus* ATCC 22959, and *Neurospora sp.*) isolated from Indonesian
344 fermented peanut press cake used. However, in this study, phytase activity in the fermented samples
345 might have originated from the yeast and the phytase inherent in the GNM.

346 **5. Conclusion**

347 Soaking and fermentation (S-TF or L-TF) were better tools for enhancing the nutritional
348 composition of GNM and CSM by improving the crude protein, crude lipid contents and amino acid
349 profile. Effective reduction of gossypol was achieved by AC while SK and L-TF were found to
350 efficiently reduce phytic acid content from CSM and GNM. However, further studies should
351 investigate combined processing techniques to completely remove gossypol and phytic acid in the
352 tested oilseed by-products.

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358 **Conflict of interest**

359 We declare that we have no financial and personal relationships with other people or
360 organizations that can inappropriately influence our work, there is no professional or other personal
361 interest of any nature or kind in any product, service and/or company that could be construed as
362 influencing the content of this paper.

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519 **Tables**520 **Table 1.**

521 Proximate composition (g/kg DM, n=3) of unprocessed and processed cottonseed meal (CSM),
 522 groundnut meal (GNM) and groundnut husk (GH).

Item	Processing technique					SEM	P-value
	UP	AC	SK	S-TF	L-TF		
CSM							
Dry matter	903.90 ^a	906.80 ^b	927.25 ^b	946.05 ^b	953.65 ^b	0.04	<0.0001
Crude protein	418.95 ^a	380.80 ^a	447.15 ^a	457.20 ^a	463.45 ^b	1.44	0.0033
Crude lipid	105.35 ^a	110.00 ^a	131.45 ^b	120.25 ^b	127.70 ^b	0.29	0.0003
Ash	80.50 ^a	80.45 ^a	53.50 ^b	54.55 ^b	38.90 ^b	0.05	<0.0001
Phosphorous	13.45 ^a	13.00 ^b	8.90 ^b	9.15 ^b	5.50 ^b	0.01	<0.0001
GNM							
Dry matter	937.25 ^a	921.10 ^b	940.20 ^b	960.85 ^b	970.15 ^b	0.05	<0.0001
Crude protein	415.35 ^a	416.40 ^a	507.95 ^b	528.75 ^b	526.50 ^b	0.29	<0.0001
Crude lipid	276.50 ^a	279.30 ^a	311.60 ^b	291.00 ^b	308.95 ^b	0.08	<0.0001
Ash	120.95 ^a	119.25 ^b	45.85 ^b	50.50 ^b	46.70 ^b	0.02	<0.0001
Phosphorous	5.15 ^a	5.00 ^a	3.35 ^b	2.95 ^b	2.20 ^b	0.01	<0.0001
GH							
Dry matter	929.70 ^a	930.75 ^a	936.40 ^b	952.40 ^b	962.15 ^b	0.05	<0.0001
Crude protein	193.35 ^a	192.65 ^a	198.50 ^b	195.95 ^a	195.70 ^a	0.12	0.0099
Crude lipid	295.15 ^a	298.20 ^a	328.75 ^b	321.95 ^b	316.50 ^b	0.27	<0.0001
Ash	38.95 ^a	39.20 ^a	30.35 ^b	32.40 ^b	31.90 ^b	0.01	<0.0001
Phosphorous	2.20 ^a	2.15 ^a	1.55 ^b	1.70 ^b	1.65 ^b	0.01	0.0002

523 UP = unprocessed; AC = autoclaving; SK = soaking; S-TF = short-term fermentation; L-TF = long-
 524 term fermentation; SEM = Pooled standard error of means.

525 ^{a, b}Mean values within a row without a common lowercase superscript differ ($P < 0.05$).

526 **Table 2.**

527 Amino acid profile of cottonseed meal (CSM) before and after autoclaving (AC), soaking (SK),
 528 short-term fermentation (S-TF) and long-term fermentation (L-TF) processes.

Item	Processing technique					SEM	P-value
	UP	AC	SK	S-TF	L-TF		
EAA, g/100 g DM							
Arginine	3.64 ^a	3.97 ^a	4.67 ^b	4.44 ^b	4.22 ^b	0.20	0.0071
Histidine	0.85	1.00	1.14	1.14	1.11	0.11	0.0646
Isoleucine	1.04 ^a	1.27 ^a	1.46 ^b	1.40 ^a	1.09 ^a	0.14	0.0444
Leucine	2.09 ^a	2.36 ^a	2.74 ^b	2.65 ^b	2.64 ^b	0.15	0.0127
Lysine	1.30 ^a	1.25 ^a	1.49 ^b	1.56 ^b	1.53 ^b	0.11	0.0002
Methionine	0.45 ^a	0.40 ^a	0.64 ^b	0.61 ^b	0.65 ^b	0.04	0.0019
Phenylalanine	1.81 ^a	2.08 ^a	2.40 ^b	2.43 ^b	2.31 ^b	0.16	0.0150
Threonine	1.22 ^a	1.31 ^a	1.55 ^b	1.56 ^b	1.59 ^b	0.05	0.0004
Valine	1.42 ^a	1.71 ^b	1.97 ^b	1.88 ^b	1.61 ^a	0.09	0.0027
TEAA	13.8 ^a	15.35	18.05	17.65	16.75		
NEAA, g/100 g DM							
Alanine	1.92 ^a	2.11 ^a	2.49 ^a	2.56 ^b	2.91 ^b	0.23	0.0142
Aspartic acid	2.79	2.59	2.92	2.95	3.07	0.20	0.1379
Cysteine	0.76 ^a	0.65 ^a	1.01 ^b	0.94 ^a	1.03 ^b	0.09	0.0109
Glutamic acid	6.46	6.24	7.03	7.01	7.07	0.54	0.2905
Glycine	1.45 ^a	1.60 ^a	1.78 ^b	1.77 ^b	1.84 ^b	0.07	0.0034
Hydroxyproline	0.07	0.07	0.07	0.08	0.07	0.01	0.5673
Proline	1.30 ^a	1.38 ^a	1.61 ^b	1.53 ^a	1.67 ^b	0.09	0.0217
Serine	1.41 ^a	1.64 ^a	1.97 ^b	1.77 ^b	2.13 ^b	0.10	0.0028
Tyrosine	1.03 ^a	1.15 ^a	1.41 ^b	1.39 ^b	1.39 ^b	0.07	0.0036
TNEAA	17.19	17.43	20.29	20.01	21.23		
TAA, g/100 g DM	31.03	32.76	38.34	37.66	36.21		
TEAA:TNEAA ratio	45:55	47:53	47:53	47:53	44:56		
TAA, % of crude protein	74.03	86.03	85.74	82.38	82.02		

529 UP = unprocessed; EAA = essential amino acids; TEAA = total essential amino acids; NEAA =
 530 non-essential amino acids; TNEAA = total non-essential amino acids; TAA = total amino acids;
 531 SEM = Pooled standard error of means.

532 ^{a, b}Mean values within a row without a common lowercase superscript differ ($P < 0.05$).

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538**Table 3.**539 Amino acid profile (g/100 g DM) of groundnut meal (GNM) before and after autoclaving (AC),
540 soaking (SK), short-term fermentation (S-TF) and long-term fermentation (L-TF) processes.

Item	Processing technique					SEM	P-value
	UP	AC	SK	S-TF	L-TF		
EAA, g/100 g DM							
Arginine	4.44 ^a	4.27 ^a	5.43 ^b	5.57 ^b	5.71 ^b	0.36	0.0089
Histidine	0.84 ^a	0.81 ^a	1.02 ^b	1.18 ^b	1.23 ^b	0.06	0.0006
Isoleucine	1.37 ^a	1.36 ^a	1.68 ^b	1.79 ^b	1.76 ^b	0.09	0.0036
Leucine	2.63 ^a	2.62 ^a	3.22 ^b	3.44 ^b	3.39 ^b	0.14	0.0012
Lysine	0.98	0.92	1.06	1.15	1.11	0.08	0.0772
Methionine	0.33 ^a	0.32 ^a	0.39 ^a	0.43 ^b	0.45 ^b	0.04	0.0191
Phenylalanine	2.07 ^a	2.04 ^a	2.60 ^b	2.70 ^b	2.78 ^b	0.19	0.0094
Threonine	1.11 ^a	1.09 ^a	1.37 ^b	1.36 ^b	1.49 ^b	0.08	0.0046
Valine	1.64 ^a	1.73 ^a	2.00 ^a	2.18 ^b	2.14 ^b	0.14	0.0124
TEAA	15.41	15.16	18.77	19.81	20.05		
NEAA, g/100 g DM							
Alanine	2.30 ^a	2.32 ^a	2.74 ^b	3.17 ^b	3.71 ^b	0.16	0.0003
Aspartic acid	4.21	4.18	4.29	5.08	4.39	0.51	0.2584
Cysteine	0.68 ^a	0.63 ^a	0.79 ^b	0.87 ^b	0.90 ^b	0.04	0.0012
Glutamic acid	7.42 ^a	7.33 ^a	7.52 ^a	8.95 ^b	7.94 ^a	0.53	0.0490
Glycine	2.10 ^a	2.04 ^a	2.38 ^a	2.43 ^a	2.47 ^b	0.15	0.0192
Hydroxyproline	0.13 ^a	0.13 ^a	0.16 ^a	0.17 ^b	0.20 ^b	0.01	0.0020
Proline	1.75 ^a	1.72 ^a	2.07 ^b	2.23 ^b	2.19 ^b	0.07	0.0006
Serine	1.98 ^a	1.89 ^a	2.14 ^a	2.52 ^b	2.57 ^b	0.08	0.0003
Tyrosine	1.70 ^a	1.66 ^a	2.09 ^a	2.17 ^b	2.26 ^b	0.15	0.0110
TNEAA	22.27	21.90	24.18	27.59	26.63		
TAA, g/100 g DM	37.68	37.06	42.95	47.40	46.68		
TEAA:TNEAA ratio	41:59	41:59	44:56	42:58	43:57		
TAA, % of crude protein	90.74	88.98	84.62	89.61	88.63		

541 UP = unprocessed; EAA = essential amino acids; TEAA = total essential amino acids; NEAA =
542 non-essential amino acids; TNEAA = total non-essential amino acids; TAA = total amino acids;
543 SEM = Pooled standard error of means.544 ^{a, b}Mean values within a row without a common lowercase superscript differ ($P < 0.05$).

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549 **Table 4.**
550 Amino acid profile of unprocessed and processed groundnut husk (GH).

Parameter	Processing technique		SEM	P-value
	UP	AC		
EAA, g/100 g DM				
Arginine	1.39	1.43	0.07	0.5160
Histidine	0.41	0.46	0.04	0.0891
Isoleucine	0.53 ^a	0.56 ^b	0.01	0.0069
Leucine	0.96	0.96	0.01	0.6626
Lysine	0.60 ^a	0.45 ^b	0.05	0.0128
Methionine	0.13 ^a	0.08 ^b	0.00	<0.0001
Phenylalanine	0.69	0.78	0.05	0.0796
Threonine	0.51	0.50	0.02	0.6555
Valine	0.62	0.64	0.01	0.3235
TEAA	5.84	5.86		
NEAA, g/100 g DM				
Alanine	1.37 ^a	1.15 ^b	0.06	0.0062
Aspartic acid	1.37 ^a	0.94 ^b	0.21	0.0431
Cysteine	0.26 ^a	0.34 ^b	0.01	0.0039
Glutamic acid	2.23 ^a	1.65 ^b	0.29	0.0492
Glycine	2.15	2.26	0.08	0.1000
Hydroxyproline	0.37	0.39	0.02	0.4704
Proline	0.68	0.66	0.02	0.1069
Serine	1.06	1.09	0.05	0.5253
Tyrosine	0.68	0.72	0.04	0.2649
TNEAA	10.17	9.21		
TAA, g/100 g DM	16.01	15.07		
TEAA:TNEAA ratio	36:64	39:61		
TAA, % of crude protein	82.89	78.23		

551 UP = unprocessed; AC = autoclaving; EAA = essential amino acids; TEAA = total essential amino
552 acids; NEAA = non-essential amino acids; TNEAA = total non-essential amino acids; TAA = total
553 amino acids; SEM = Pooled standard error of means.

554 ^{a, b}Mean values within a row without a common lowercase superscript differ ($P < 0.05$).

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Table 5.

563 Nitrogen contents (g/kg DM) and their changes (% , in brackets) of unprocessed and processed
564 cottonseed meal (CSM), groundnut meal (GNM) and groundnut husk (GH).

Item	Processing technique					SEM	P-value
	UP	AC	SK	S-TF	L-TF		
CSM							
TN	67.00	60.95 (-9.02)	71.55 (6.79)	73.15 (9.18)	74.15 (10.67)	0.23	< 0.0001
TAA-N	49.70	52.40 (5.43)	61.35 (23.44)	60.25 (21.22)	60.75 (22.23)	0.27	< 0.0001
NPN	17.40	8.55 (-50.86)	10.20 (-41.38)	12.90 (-25.86)	13.40 (-22.99)	0.45	0.0124
GNM							
TN	66.40	66.60 (0.30)	81.30 (22.44)	84.60 (27.41)	84.20 (26.81)	0.05	< 0.0001
TAA-N	60.30	59.30 (-1.66)	68.80 (14.10)	75.80 (25.71)	74.70 (23.88)	0.24	< 0.0001
NPN	6.20	7.40 (19.35)	12.50 (101.61)	8.80 (41.94)	9.50 (53.23)	0.28	0.0047
GH							
TN	30.90	30.80 (-0.32)	31.80 (2.91)	31.40 (1.62)	31.40 (1.62)	0.06	< 0.0001
TAA-N	25.70	24.10 (-6.23)	-	-	-	1.39	0.0013
NPN	5.30	6.70 (26.42)	-	-	-	0.03	0.0025

565 UP = unprocessed; AC = autoclaving; SK = soaking; S-TF = short-term fermentation; L-TF = long-
566 term fermentation; SEM = Pooled standard error of means; TN = total nitrogen; TAA-N = total amino
567 acid nitrogen; NPN = non-protein nitrogen.

568 ^{a, b}Mean values within a row without a common lowercase superscript differ ($P < 0.05$).

569 **Table 6.**

570 Gossypol content (mg/g DM), Phytic acid content (g/100 g) and loss (% , in bracket) of unprocessed
 571 and processed cottonseed meal (CSM), groundnut meal (GNM) and groundnut husk (GH).

Item	Processing technique					SEM	P-value
	UP	AC	SK	S-TF	L-TF		
Gossypol							
CSM	0.29 ^a	0.19 ^b (34.48)	0.21 ^b (27.59)	0.24 ^b (17.24)	0.25 ^a (13.79)	0.02	0.0043
GNM	0.31 ^a	0.28 ^a (9.68)	0.21 ^b (32.26)	0.17 ^b (45.16)	0.20 ^b (35.48)	0.03	0.0041
GH	1.75 ^a	0.93 ^b (46.86)	0.67 ^b (61.71)	0.58 ^b (66.86)	0.61 ^b (65.14)	0.14	0.0005
Phytic acid							
CSM	3.84 ^a	3.53 ^a (8.07)	2.23 ^b (41.93)	2.25 ^b (41.1)	1.08 ^b (71.86)	0.13	< 0.0001
GNM	1.40 ^a	1.19 ^a (15.00)	0.85 ^b (39.29)	0.73 ^b (47.86)	0.43 ^b (69.29)	0.10	0.0003
GH	0.41 ^a	0.41 ^a (0.00)	0.28 ^b (31.71)	0.35 ^a (14.63)	0.29 ^b (29.27)	0.03	0.0085

572 UP = unprocessed; AC = autoclaving; SK = soaking; S-TF = short-term fermentation; L-TF = long-
 573 term fermentation; SEM = Pooled standard error of means.

574 ^{a, b}Mean values within a row without a common lowercase superscript differ ($P < 0.05$).

575 **Figures**

576

577 **Fig. 1.** Changes in essential amino acid contents in cottonseed meal after autoclaving (AC, A),
578 soaking (SK, B), short-term fermentation (S-TF, C) and long-term fermentation (L-TF, D). Arg =
579 arginine; His = histidine; Ile = isoleucine; Leu = leucine; Lys = lysine; Met = methionine; Phe =
580 phenylalanine; Thr = threonine; Val = valine.

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582 **Fig. 2.** Changes in essential amino acid contents in groundnut meal after autoclaving (AC, A),
583 soaking (SK, B), short-term fermentation (S-TF, C) and long-term fermentation (L-TF, D). Arg =
584 arginine; His = histidine; Ile = isoleucine; Leu = leucine; Lys = lysine; Met = methionine; Phe =
585 phenylalanine; Thr = threonine; Val = valine.

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588 **Fig. 3.** Changes in essential amino acid contents in groundnut husk after autoclaving (AC). Arg =
589 arginine; His = histidine; Ile = isoleucine; Leu = leucine; Lys = lysine; Met = methionine; Phe =
590 phenylalanine; Thr = threonine; Val = valine.

