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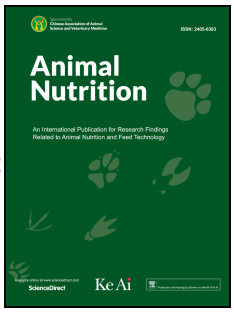

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Processing techniques of selected oilseed by-products of potential use in animal feed: Effects on proximate nutrient composition, amino acid profile and antinutrients

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ABSTRACT

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

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

1. Introduction

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

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

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

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

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

2. Materials and methods

2.1. Sources and preparation of raw materials

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

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

2.2. Processing procedures

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

2.2.1. Autoclaving

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

2.2.2. Soaking

The samples of CSM, GNM and GH were transferred to 2 L glass jars. Tap water was added at a ratio of 1:10 (w/V). The samples were allowed to soak at room temperature for 12 h with intermittent stirring every 4 h after which the water was decanted. The samples were transferred onto a fine meshed cloth (100 μm) and squeezed, to remove as much of the water as possible. The residual meal was spread on a tray and oven dried (Memmert, UN110) at 40 ºC to constant weight. After drying and cooling to room temperature, the samples were finely ground, sealed in polythene bags, and stored at -20ºC 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 (Memmert UN110). Ash
117 content was determined by incineration of the samples for 6 h at 550 °C in a muffle furnace
118 (Hareaus Instruments K1252). Crude protein content was determined by the Kjeldahl method
119 (FOSS Kjeltec 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

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

3. Results

3.1. Proximate composition

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

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

3.4. Anti-nutritional factors

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

4. Discussion

4.1. Proximate composition

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

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

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

4.2. Amino acid composition

Soaking, S-TF and L-TF improved the amino acid profile of CSM and GNM compared to unprocessed samples. Generally, water-soluble amino acids are expected to be lost through SK yet, the non-deleterious effect of SK on the amino acid profile could be linked to the plant by-products' composition of higher proportions of insoluble amino acids that may primarily function as structural parts of the plant (Wade, 2009). A possible explanation for the significant increases in most of the amino acids observed for the fermented and soaked samples could be that the oilseed by-products in their raw state contained sufficient quantities of NPN to meet the yeast's nitrogen requirements. Alternatively, the amino acid contents of the raw samples were protein bound, and not available for 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 bicolour* 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 cerevisae*) 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 cerevisae* (88.5%), *Aspergillus niger* (85.2%) and
317 *Aspergillus terricola* (82.9%). These reductions in free gossypol were attributed to microbial or

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

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

363

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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	
	UP	AC	SK	S-TF	L-TF		*P*-value
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 ($P < 0.05$).

Table 3.

Amino acid profile (g/100 g DM) of groundnut meal (GNM) before and after autoclaving (AC), 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		

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

[a, b]Mean values within a row without a common lowercase superscript differ ($P < 0.05$).

Table 4.

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		

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

[a, b]Mean values within a row without a common lowercase superscript differ ($P < 0.05$).

Table 5.

Nitrogen contents (g/kg DM) and their changes (%, in brackets) of unprocessed 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		
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

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

[a, b]Mean values within a row without a common lowercase superscript differ ($P < 0.05$).

Table 6.

Gossypol content (mg/g DM), Phytic acid content (g/100 g) and loss (%, in bracket) of unprocessed 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

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

[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.

581

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.

586

587

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.