Effect of Domestic Processing Methods on Chemical Composition, In vitro Digestibility of Protein and Starch and Functional Properties of Bambara Groundnut (Voandzeia subterranea) Seed

Abu El-Gasim Ahmed Yagoub and Abdalla Abdelsamad Abdalla

Faculty of Agriculture, University of Zalingei, Sudan.

Faculty of Agriculture and Natural Resources, University of Kordofan, Sudan.

Abstract: Comparative effects of domestic processing methods on chemical composition, in vitro digestibility of protein and starch in bambara groundnut (Voandzeia subterranea) were studied. Protein fractions, protein solubility indices and some functional properties in water and 1 M NaCl extracts from flour samples were also assessed. The different methods showed varied deviation of nutrients and antinutrients from the raw seeds. Germination significantly increased protein content and decreased starch level. Thermal treatments, specially roasting, were more effective in reduction of total polyphenols. Germination was the best method to eliminate phytic acid and to increase in vitro protein digestibility. Cooking, in particular of the presoaked seeds, was most effective in improving in vitro starch digestibility. The results also indicated that domestic processing methods changed N solubility in water and 1 M NaCl. The foam volume from the flours indicates a significant decrease as a result of soaking, germination, cooking and roasting. Addition of 1 M NaCl did not show improvement in foaming capacity of the flours. Germination (4 and 6 days) significantly improved foaming stability in water and 1 M NaCl. Thermal treatments significantly decreased stability in each solvent. The emulsification stability in water and 1 M NaCl of almost all samples studied was significantly decreased.

Key words: Voandzeia subterranea, domestic processing, chemical composition, in vitro digestibility of protein and starch, functional properties

INTRODUCTION

Bambara groundnut (Voandzeia subterranea) is a tropical food legume in Sudan and other tropical areas. Due to the high price of meat and fish, much importance is now placed on grain legumes as a source of proteins in all the developing countries. Legumes are rich not only in proteins, but in other nutrients such as starch[28]. The nutritional value of legume seeds is restricted by the presence of antinutrients such as polyphenols, phytic acid and enzyme inhibitors[19,41].

A review of literature reveals limited information on physicochemical properties of bambara groundnut seeds[14,33]. Processing methods, such as soaking, germination and cooking has been reported to improve the nutritional and functional properties of legumes[26,4,2,18,38]. In western Sudan, bambara groundnut is not a part of the main dish in the table of the local people. It’s consumed as a salt-boiled snack food. Therefore, investigation of the effects of different domestic processing methods on the nutritional and functional attributes of this legume may draw sight of the local people toward this crop. This may broaden the scope of its utilization in the food system.

The aim of this study was to assess the efficiency of processing methods, such as soaking, germination, ordinary cooking and roasting on the changes in chemical composition of bambara groundnut seed in relation to in vitro digestibility of protein and starch and functional properties.

MATERIALS AND METHODS

Bambara groundnut seeds (Voandzeia subterranea) cultivated in Zalingie (Western Darfur, Sudan) were employed for this study.

Domestic Processing of Seeds: Bambara groundnut seeds were graded, cleaned and divided into five unequal batches. One batch without any treatment served as control (designated C). Two batches were cooked in a boiling distilled water and 2% NaCl solution (designated UNSCw and UNSCs, respectively) until softened on squeeze between fingers (~ 2 hours). The cooked seeds were drained and then dried in an oven at 60 °C for 24 hours. Another batch was dry
roasted on a hot plate (~ 230 °C) to a creamy-colored appearance. The seeds were cooled by aeration. The seed had an initial moisture content of 6.0% before roasting, and the final moisture content was 1.5%. The larger batch was soaked in the dark in a solution of sodium azide (0.005 M) overnight at room temperature (~ 27 °C). The soaked seeds were then drained and divided into five batches. One batch of the soaked seeds was subjected to ordinary cooking in distilled water as before (~ 1 hour). Three equal batches of the soaked seeds were transferred to trays containing wet sandy soils and germinated at room temperature (~ 27 °C) for 2, 4 and 6 days. The soaked, soaked-cooked and germinated seeds, designated S, SC, G2, G4 and G6, were dried as before.

**Preparation of Flour:** All nine batches of raw and processed bambara groundnut seeds were powdered (60 mesh screen), bottled and kept at 4 °C for all further studies.

**Chemical Analysis:**

**Proximate analysis:** Lipids, ash, total carbohydrates and total nitrogen (micro-Kjeldahl) were determined according to AOAC[1]. Protein was calculated as N X 6.25. Moisture content was determined by drying samples at 105 °C overnight[2] and then dry matter was calculated. Crude fiber content was determined by acid/alkali digestion method of Southgate[3].

**Nonprotein nitrogen:** Nonprotein nitrogen (NPN) was determined by Paredez-Lopez and Harry[4]. It was measured as nitrogen soluble in 12% trichloroacetic acid (TCA).

**Soluble carbohydrates:** A soluble carbohydrate of the samples was determined according to the method described by Paredez-Lopez and Harry[4]. They were quantified using the phenol-sulfuric acid method of Dubois et al.[5] with glucose as a standard.

**Starch:** A starch content of samples was determined according to the method of Faithful[6], with slight modification. Hundred milligrams defatted flour, in a beaker, were extracted with 10 ml ethanol (10% v/v) by continuous shaking for 30 minutes to remove soluble carbohydrates. The mixture was centrifuged at 3000 rpm for 5 minutes at room temperature (~ 27 °C), and the supernatant was decanted. The residue was washed thoroughly with 1 M H2SO4 solution and then centrifuged. Fifteen milliliters of 1 M H2SO4 were added to the clean residue, covered and heated in a boiling water bath for 45 minutes. After cooling, the contents were washed into 100 ml volumetric flask and the volume made up to mark. After settlement, 10 ml aliquot was taken and made up to 100 ml in a volumetric flask. The glucose of the hydrolysate was quantified using the Dubois et al.[5] method. The starch was expressed as:

\[ \text{Starch \%} = \text{glucose \%} \times 0.9 \]

**Polyphenols:** The Folin-Denis reagent method described by Alonzo et al.[7] was used with some modifications. Total phenols were extracted in a sample of 1 g flour with 100 ml 0.3% oxalic acid by mechanical shaking for 30 minutes. One milliliter of the supernatant, obtained after centrifugation (3000 rpm for 15 minutes) at room temperature, was diluted to 8 ml with distilled water. Then 0.5 ml Folin-Denis reagent was added, shaken and 3 minutes later 1.5 ml of saturated sodium carbonate was added. After an hour the absorbance was read at 760 nm. Tannic acid was used as a reference standard.

**Phytic acid:** Phytates of the samples were determined according to the method of Wheeler and Ferrel[8]. Phytate was extracted from samples with 3% trichloroacetic acid (TCA) solution containing 10% (w/v) sodium sulfate, and precipitated using ferric chloride (0.2% Fe3+). The iron recovered by boiling with NaOH and then with HNO3 was quantified by reading the intensity of the colored complex formed, after addition of potassium thiocyanate, in a Jenway 6305 a spectrophotometer at 480 nm. The iron content was calculated from ferri nitrate standard curve and the data extrapolated to phytic acid. A ratio of iron to phosphorus of 4:6 was assumed.

**In vitro protein digestibility:** In vitro protein digestibility (IVPD) of the samples was measured according to the method developed by Saunders et al.[9] in which a peptin-pancreatin system of digestion was used in the determinations. The digestible protein was analyzed for nitrogen using the micro Kjeldahl procedure[9] and expressed as a percent of the total N.

**In Vitro Starch Digestibility:** The in vitro starch digestibility (IVSD) was determined in flours (50 mg/ml of 0.2 M phosphate buffer, pH 6.9) after amylolysis with 0.5 ml pancreatic amylase (1260 U/ml) suspension (0.4 mg/ml of 0.2 M phosphate buffer, pH 6.9) at 37 °C for 4 hours according to the method of Singh et al.[10]. At the end of the incubation period, 2 ml of 1% 3,5-dinitrosalicylic acid reagent were added and the mixture boiled for 5 minutes. After cooling, the mixture was completed to 20 ml with distilled water and the absorbance of the filtered solution was read at 550 nm with maltose used as standard. The IVSD was expressed as mg of maltose...
per gram of sample on a dry weight basis.

**Osborne Classification of Proteins:** The proteins from the flours of the samples were fractionated according to the technique of Osborne as described by Abd Elal et al.[1] using distilled water, 1 M NaCl, 70% ethanol and 0.2% NaOH solutions for albumins, globulins, prolamins and glutelins, respectively. The nitrogen content of each fraction was determined using the micro-Kjeldahl procedure[9]. The residue left after extraction was also analyzed for nitrogen content. Each fraction was expressed as a percent of the total nitrogen.

**Nitrogen Solubility:** Nitrogen solubility, both in water and 1 M NaCl, of samples was determined following the method described by Prakash[24]. The water soluble nitrogen in the flour was extracted by rotary shaking with distilled water at 1:10 solute to solvent ratio for 1 hour, at room temperature. The slurry was centrifuged at 3000 rpm for 30 minutes at room temperature. The nitrogen value of the supernatant obtained was determined according to micro-Kjeldahl procedure[9] and expressed as milligram protein per milliliter solution.

**Nitrogen Solubility Profile:** A Nitrogen solubility profile of samples was determined by extraction in water and 1 M NaCl solution over a pH range of 1 - 12 according to the method described by Quinn and Beuchat[25]. A 2% (w/v) flour suspension was shaken for 10 minutes before the desired pH was maintained by addition of 2 N HCl or 2 N NaOH over a 60 minutes period of constant shaking at room temperature. The suspension was centrifuged at 3000 rpm, at room temperature, for 20 minutes. The soluble nitrogen in supernatant was determined by Kjeldahl procedure[9]. The percentage of the soluble nitrogen was calculated and plotted against corresponding recorded pH values. The pH range of minimum extractability was determined.

**Functional properties measurements:**

- **Water absorption capacity:** Water absorption capacity (WAC) of samples was determined according to the method of Lin et al.[26] with a modification described by Wang and Kinsella[19]. A 10% flour suspension was stirred in a 50-ml centrifuge tube using a glass rod for 2 minutes at room temperature. After 30 minutes shaking the tube was centrifuged at 3700 for 25 minutes at room temperature. The freed water was carefully decanted in a graduated measuring cylinder and the volume recorded. The WAC was corrected for the loss of soluble components and expressed as milliliters water retained by 1 gram flour.

- **Fat Absorption Capacity:** Fat absorption capacity (FAC) of samples was measured by the method of Lin et al.[26]. The FAC expressed as ml oil retained per 1 gram flour.

- **Foaming Capacity and Foam Stability:** Foaming capacity and foam stability of samples were determined following the method described by Venktesh and Prakash[24]. A 3% flour suspension was stirred in a kitchen blender for 6 minutes, transferred to a 500 ml measuring cylinder, and the volume of foam at 30 seconds was calculated, and the increase in volume was expressed as a percent foam capacity. The foam stability was determined by measuring the decrease in volume of foam as a function of time up to a period of 30 minutes.

- **Emulsification Activity and Emulsion Stability:** Emulsification activity (EA) was determined according to the method described by Venktesh and Prakash[24]. Thirty milliliter of distilled water and 10 ml of refined peanut oil were added to 1.5 g of flour, and the mixture was stirred. The contents were homogenized in a Virtis homogenizer at 2000 g for 1 minute. An aliquot of 0.10 ml was drawn immediately and at regular intervals of time from the bottom of the container and diluted to 10 ml with 0.1% sodium dodecylsulfate and the absorbance was measured at 500 nm in a Jenway 3536 spectrophotometer. To measure the absorbance, the emulsion was diluted so as to read within 1.0 absorbance in a spectrophotometer the reading is multiplied by a dilution factor, and the resulting absorbances are plotted on the Y axis. A graph of absorbance against time was plotted. The time for the initial absorbance (emulsification activity) to decrease by half was recorded as emulsion stability.

**Statistical Analysis:** The results are given as means of triplicate samples. Where appropriate statistical analysis of variance (ANOVA) was done to determine the significance differences among means followed by Duncan’s Multiple range test when the F-test demonstrated significance[12]. The statistically significant difference was defined as p ≤ 0.01.

**RESULTS AND DISCUSSIONS**

**Chemical Composition:** Table 1 shows results of chemical composition of bambara groundnut seeds as a function of domestic processing methods. Soaking (12 hours), germination (2-6 days) of presoaked seeds, cooking of dry seeds (in water and 2% Na Cl), cooking of presoaked seeds and roasting resulted in slight deviation of nutrients from the raw seeds. The difference in nutrient contents is due to leaching of
soluble components during soaking and cooking and as a consequence of enzyme activities during germination\cite{13,21,28}. The results agree with those found by Obizoba\cite{22} and Camacho et al.\cite{8} in some legume seeds.

**Starch and Soluble Carbohydrates:** Table 1 also shows that starch content of Bambara groundnut seed (56.58\%) was significantly ($p \leq 0.01$) increased, concurrently with a significant ($p \leq 0.01$) decrease in soluble carbohydrates during soaking of the seeds. Germination for four and six days resulted in a significant ($p \leq 0.01$) increase in soluble carbohydrates and a decrease in the level of starch (Table 1). The results agree well with the notable increase in starch digestibility found during germination (Table 2), presumably caused by starch digestion through amylolytic enzymes\cite{21}. On the other hand, soaking, cooking and roasting did not show profound changes in the level of starch and soluble carbohydrates.

**Total polyphenols and Phytic acid:** Data on polyphenol and phytic acid contents of raw and processed Bambara groundnut seeds are summarized in Table 2.

Soaking (12 hours), germination (2 – 6 days), cooking of presoaked and dry seeds (in water and 2% NaCl solution) and roasting significantly ($p \leq 0.01$) reduced the level of polyphenols. The data agree with those found by Camacho et al.\cite{8}, Savelkoul et al.\cite{30}, Bakr\cite{4} and Alonso et al.\cite{2} in other legume seeds. Compared to the raw seeds, a significant ($p \leq 0.01$) reduction in the level of polyphenols was observed after 12 hours soaking (18.43\%) as well as after 2, 4 and 6 days of germination (27.19, 31.31 and 34.56\%, respectively). Heat treated samples had the lowest polyphenol content. Heat degradation and/or leaching of these molecules as well as change in their chemical reactivity or the formation of insoluble complexes could explain the significant reduction of these antinutrients by thermal methods\cite{17,24,2}.

The phytic acid content of Bambara groundnut seed (811.00 mg/100g) was decreased significantly ($p \leq 0.01$) by soaking, germination and cooking of the presoaked seeds (Table 2). Germination was more effective in lowering the level of phytic acid. Leaching out effect during hydration\cite{5} and the increase in phytase activity during germination\cite{6,2} could be responsible for the loss of phytate. Evidences indicate the

Stability of phytic acid to cooking heat\cite{23,33}. Soaking in water followed by cooking has been reported to result in substantial loss in phytate\cite{6,27}.

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**Table 1:** Chemical composition of raw and processed Bambara groundnut (Percent*).

<table>
<thead>
<tr>
<th>Sample**</th>
<th>Total protein</th>
<th>Crude fat</th>
<th>Crude fiber</th>
<th>Ash</th>
<th>Carbohydrates#</th>
<th>Starch</th>
<th>Soluble carbohydrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>22.70*</td>
<td>5.00*</td>
<td>3.72*</td>
<td>3.76*</td>
<td>65.00*</td>
<td>56.85*</td>
<td>5.43*</td>
</tr>
<tr>
<td></td>
<td>(0.22)</td>
<td>(0.28)</td>
<td>(0.15)</td>
<td>(0.03)</td>
<td>(0.21)</td>
<td>(0.14)</td>
<td>(0.10)</td>
</tr>
<tr>
<td>S</td>
<td>22.21*</td>
<td>4.95*</td>
<td>3.74*</td>
<td>3.68*</td>
<td>65.42*</td>
<td>58.31*</td>
<td>5.23*</td>
</tr>
<tr>
<td></td>
<td>(0.40)</td>
<td>(0.40)</td>
<td>(0.15)</td>
<td>(0.04)</td>
<td>(0.07)</td>
<td>(0.05)</td>
<td>(0.08)</td>
</tr>
<tr>
<td>G²</td>
<td>23.16*</td>
<td>4.97*</td>
<td>3.98*</td>
<td>3.57*</td>
<td>65.32*</td>
<td>57.47*</td>
<td>5.37*</td>
</tr>
<tr>
<td></td>
<td>(0.18)</td>
<td>(0.03)</td>
<td>(0.14)</td>
<td>(0.04)</td>
<td>(0.19)</td>
<td>(0.07)</td>
<td>(0.10)</td>
</tr>
<tr>
<td>G⁴</td>
<td>24.10*</td>
<td>4.83*</td>
<td>3.94*</td>
<td>4.86*</td>
<td>62.29*</td>
<td>55.04*</td>
<td>6.63*</td>
</tr>
<tr>
<td></td>
<td>(0.30)</td>
<td>(0.09)</td>
<td>(0.14)</td>
<td>(0.02)</td>
<td>(0.06)</td>
<td>(0.20)</td>
<td>(0.04)</td>
</tr>
<tr>
<td>G⁶</td>
<td>24.23*</td>
<td>4.86*</td>
<td>4.39*</td>
<td>4.25*</td>
<td>62.27*</td>
<td>53.29*</td>
<td>7.56*</td>
</tr>
<tr>
<td></td>
<td>(0.34)</td>
<td>(0.09)</td>
<td>(0.13)</td>
<td>(0.03)</td>
<td>(0.07)</td>
<td>(0.08)</td>
<td>(0.06)</td>
</tr>
<tr>
<td>SC</td>
<td>22.21*</td>
<td>4.30*</td>
<td>4.53*</td>
<td>3.55*</td>
<td>65.41*</td>
<td>58.40*</td>
<td>5.25*</td>
</tr>
<tr>
<td></td>
<td>(0.20)</td>
<td>(0.03)</td>
<td>(0.12)</td>
<td>(0.02)</td>
<td>(0.06)</td>
<td>(0.14)</td>
<td>(0.07)</td>
</tr>
<tr>
<td>UNSCw</td>
<td>22.46*</td>
<td>4.53*</td>
<td>3.36*</td>
<td>3.21*</td>
<td>66.44*</td>
<td>57.64*</td>
<td>5.38*</td>
</tr>
<tr>
<td></td>
<td>(0.35)</td>
<td>(0.02)</td>
<td>(0.13)</td>
<td>(0.02)</td>
<td>(0.17)</td>
<td>(0.06)</td>
<td>(0.06)</td>
</tr>
<tr>
<td>UNSCs</td>
<td>22.48*</td>
<td>4.38*</td>
<td>3.34*</td>
<td>3.23*</td>
<td>66.57*</td>
<td>57.37*</td>
<td>5.42*</td>
</tr>
<tr>
<td></td>
<td>(0.52)</td>
<td>(0.02)</td>
<td>(0.14)</td>
<td>(0.02)</td>
<td>(0.02)</td>
<td>(0.06)</td>
<td>(0.04)</td>
</tr>
<tr>
<td>R</td>
<td>22.54*</td>
<td>4.80*</td>
<td>3.20*</td>
<td>3.82*</td>
<td>66.64*</td>
<td>57.42*</td>
<td>5.46*</td>
</tr>
<tr>
<td></td>
<td>(0.12)</td>
<td>(0.04)</td>
<td>(0.12)</td>
<td>(0.03)</td>
<td>(0.18)</td>
<td>(0.07)</td>
<td>(0.06)</td>
</tr>
</tbody>
</table>

*Means of triplicate samples. Values within parentheses are standard deviations. Means followed by different letters within a column are significantly different according to DMRT ($p \leq 0.01$). Calculations on free moisture basis.

**C:** Control Bambara groundnut seed; **S:** Soaked seeds; **G²:** 2-day old sprout; **G⁴:** 4-day old sprout; **G⁶:** 6-day old sprout; **SC:** Soaked-cooked seeds; **UNSCw:** Unsoaked-cooked seed in water; **UNSCs:** Unsoaked-cooked in 2\% NaCl. **R:** Roasted seed.

# obtained by difference.
Table 2: Phytic acid (PA), total polyphenols (TPP) and in vitro digestibility of starch (IVSD) and protein (IVPD) of raw and processed bambara groundnut seed.

<table>
<thead>
<tr>
<th>Sample**</th>
<th>PA mg/100g</th>
<th>TPP mg/100g</th>
<th>IVSD mg maltose/g</th>
<th>IVPD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>811±70</td>
<td>217±2.41</td>
<td>247.5±4.28</td>
<td>78.75±0.35</td>
</tr>
<tr>
<td>S</td>
<td>710±20</td>
<td>177±2.83</td>
<td>258.2±2.20</td>
<td>75.71±0.30</td>
</tr>
<tr>
<td>G2</td>
<td>687±30</td>
<td>158±3.26</td>
<td>255.9±1.56</td>
<td>76.92±0.40</td>
</tr>
<tr>
<td>G4</td>
<td>647±40</td>
<td>149±4.24</td>
<td>270.7±4.10</td>
<td>84.41±0.16</td>
</tr>
<tr>
<td>G6</td>
<td>566±20</td>
<td>142±2.83</td>
<td>285.0±2.83</td>
<td>83.39±0.04</td>
</tr>
<tr>
<td>SC</td>
<td>664±22</td>
<td>127±2.94</td>
<td>343.1±4.60</td>
<td>48.76±0.08</td>
</tr>
<tr>
<td>UNSCw</td>
<td>799±60</td>
<td>113±1.80</td>
<td>329.9±3.94</td>
<td>51.57±0.10</td>
</tr>
<tr>
<td>UNSCs</td>
<td>804±52</td>
<td>116±2.60</td>
<td>321.2±4.40</td>
<td>50.80±0.28</td>
</tr>
<tr>
<td>R</td>
<td>792±58</td>
<td>94±1.37</td>
<td>294.1±6.20</td>
<td>42.10±0.31</td>
</tr>
</tbody>
</table>

*Means of triplicate samples ± SD. Means followed by different letters within a column are significantly different according to DMRT (p < 0.01). Calculations on free moisture basis. Figures in the parentheses indicate the percent increase or decrease over the values of the corresponding control raw seed.

**C: Control bambara groundnut seed; S: Soaked seeds; G2: 2-day old sprout; G4: 4-day old sprout; 6-day old sprout; SC: Soaked-cooked seeds; UNSCw: Unsoaked-cooked seed in water; UNSCs: Unsoaked-cooked in 2% NaCl. R: Roasted seed.

In Vitro Protein Digestibility: Table 2 shows that in vitro protein digestibility (IVPD) of bambara groundnut seed (78.75%) was increased significantly (p ≤ 0.01) by 7.19% and 5.89% in 4-day and 6-day old sprouts, respectively. The results agree with those obtained by Savelkoul et al.[10], Jirapa et al.[11] and Waldron[12] in legume seeds. Improvement of protein digestibility after germination could be attributable to reduction of polyphenols and phytic acid (Table 2) and increase in insoluble proteins (Table 3) brought about by proteolytic activity of enzymes in the germinated seedling. The increase in the proteolytic activity of enzymes inherent in sprouting seedlings was found effective in hydrolyzing protein-polyphenol complex[11,12], hence digestibility increased.

Thermal treatments (cooking and roasting conditions) significantly (p ≤ 0.01) decreased IVPD in bambara groundnut seeds. Resistance to proteolytic degradation has been attributed to the presence of bound carbohydrates or polyphenols or to protein conformation[13,14]. Isopeptide formation during thermal treatments is also possible, especially during roasting. This may also be responsible for the lower digestibility in the heat treated samples. The results agree with the relative increase in gelulins (Table 4). Since protein digestibility has been reported to decrease with increased highly polymer gelulins[15].

In Vitro Starch Digestibility: In vitro starch digestibility (IVSD) of bambara groundnut seed (247.50 mg/g) was increased significantly (p ≤ 0.01) by soaking, germination, ordinary cooking and roasting (Table 2). The results agree with those of Alonso et al.[12] and Jirapa et al.[11]. Thermal treatments were found most effective in increasing IVSD.

The degree of starch gelatinization of the heat treated samples is higher than other ones[7] and it is thus more readily hydrolyzed. The rupture of starch granules facilitates the amyolysis[2]. Resistance to α-amyolitic degradation has been attributed to presence of polyphenols and/or to amylase inhibitors and it can be decreased by heat destruction of those molecules[7].

Nitrogen Solubility: Nitrogen solubility, in water and 1 M NaCl, of raw (control) and processed bambara groundnut seed flours is shown in Table 3. The data show that the proteins extracted in water and 1 M

fractions of the ring domestic processing methods protein complexes with carbohydrates and polyphenols, and the increase in protein denaturation have been found to reduce protein solubility\textsuperscript{[37,2]}.

**Protein Fractions:** Osborne classification of bambara groundnut seed proteins showed variation in protein fractions during domestic processing methods (Table 4). Bambara groundnut seed albumin represent the major protein fraction (72.64\%), followed by glutelin (8.60\%) and then globulin (8.20\%). Prolamin was the lowest fraction (0.84\%). Soaked and germinated samples do not show considerable changes in protein fractions if compared to the raw sample.

Compared to the control raw seed, a significant reduction in the level of albumin in the heat treated samples was observed. As a result a relative increase in glutelins and insoluble proteins was found. The variation in the protein fractions observed is the consequences of changes of the molecular mass of the different proteins. The changes in protein conformation and complexation of proteins due to heat\textsuperscript{[37,2]} may modify their solubility and they are extracted in other conditions.

### Nitrogen Solubility Profiles in Water

Nitrogen solubility profiles of the total proteins of raw and processed bambara groundnut seed flours extracted in water at pH values from 1 to 12 are shown in Figure 1a.

Total proteins of the control bambara groundnut seed flour (C) had a solubility minimum at pH 4.5 – 5.5 (i.e. isoelectric pH, PI). N solubility increased at both sides to the PI region with maximum solubility at pH 10 (82.67\%). Germination process showed a significant improvement in the amount of protein extracted at pH 1-6. A significant decrease in protein extractability at all pH values was observed in the soaked and heat treated samples. The differences in the nature and charge properties of the proteins of the control and processed samples could

\begin{table}
\centering
\caption{Protein fractions of raw and processed bambara groundnut seed (percent*).}
\begin{tabular}{lccccccc}
Sample & Albumin & Globulin & Prolamin & Glutelin & Insoluble protein & Protein recovery & NPN\#  \\
\hline
C & 72.64\% (0.80) & 8.20\% (0.28) & 0.84\% (0.05) & 8.60\% (0.03) & 11.45\% (0.10) & 101.73 & 7.84\% (0.12)  \\
S & 68.63\% (0.89) & 4.90\% (0.28) & 0.86\% (0.06) & 9.55\% (0.07) & 13.69\% (0.13) & 97.63 & 7.97\% (0.12)  \\
G2 & 70.77\% (0.50) & 5.28\% (0.07) & 6.13\% (0.16) & 8.90\% (0.32) & 9.79\% (0.16) & 100.87 & 9.15\% (0.20)  \\
G4 & 72.01\% (0.38) & 5.34\% (0.70) & 5.70\% (0.14) & 9.25\% (0.21) & 9.73\% (0.07) & 102.03 & 9.62\% (0.14)  \\
G6 & 68.10\% (0.25) & 5.46\% (0.06) & 5.70\% (0.13) & 10.34\% (0.23) & 9.70\% (0.04) & 99.30 & 9.70\% (0.18)  \\
SC & 9.27\% (0.21) & 8.63\% (0.04) & 3.43\% (0.07) & 53.75\% (0.35) & 23.48\% (0.12) & 98.56 & 8.55\% (0.22)  \\
UNSCw & 7.49\% (0.13) & 7.96\% (0.23) & 3.79\% (0.33) & 61.14\% (0.23) & 21.64\% (0.43) & 102.02 & 8.37\% (0.14)  \\
UNSCs & 7.10\% (0.28) & 8.10\% (0.20) & 3.81\% (0.03) & 61.00\% (0.28) & 21.11\% (0.06) & 101.12 & 8.35\% (0.11)  \\
R & 5.70\% (5.70) & 6.58\% (0.07) & 3.15\% (0.07) & 61.50\% (0.30) & 21.38\% (0.06) & 98.30 & 8.23\% (0.10)  \\
\end{tabular}
\end{table}

*Means of triplicate samples. Values within parentheses are standard deviations. Means followed by different letters within a column are significantly different according to DMRT (p ≤ 0.01). Calculations on free moisture basis.

** C: Control bambara groundnut seed; S: Soaked seeds; G2: 2-day old sprout; G4: 4-day old sprout; 6-day old sprout; SC: Soaked-cooked seeds; UNSCw: Unsoaked-cooked seed in water; UNSCs: Unsoaked-cooked in 2% NaCl. R: Roasted seed.

# NPN: Nonprotein nitrogen

\begin{table}
\centering
\caption{Water absorption capacity (WAC) and fat absorption capacity (FAC) of raw and processed bambara groundnut seed.}
\begin{tabular}{lcccc}
Sample & WAC\# ml/g & FAC\# ml/g  \\
\hline
C & 4.87\% ± 0.04 & 3.90\% ± 0.03  \\
S & 1.50\% ± 0.01 & 1.00\% ± 0.01  \\
G2 & 1.54\% ± 0.02 & 1.00\% ± 0.02  \\
G4 & 1.61\% ± 0.03 & 1.00\% ± 0.02  \\
G6 & 1.76\% ± 0.03 & 1.00\% ± 0.01  \\
SC & 3.05\% ± 0.02 & 0.45\% ± 0.02  \\
UNSCw & 2.73\% ± 0.01 & 3.50\% ± 0.02  \\
UNSCs & 2.67\% ± 0.02 & 3.50\% ± 0.01  \\
R & 2.16\% ± 0.03 & 3.50\% ± 0.03  \\
\end{tabular}
\end{table}

*Means of triplicate samples ± SD. Means followed by different letters within a column are significantly different according to DMRT (p ≤ 0.01). ** C: Control bambara groundnut seed; S: Soaked seeds; G2: 2-day old sprout; G4: 4-day old sprout; 6-day old sprout; SC: Soaked-cooked seeds; UNSCw: Unsoaked-cooked seed in water; UNSCs: Unsoaked-cooked in 2% NaCl. R: Roasted seed.

# Water absorbed was corrected for the soluble components.

NaCl solution of the control seed (17.11 and 17.70 mg/mL, respectively) were significantly (p ≤ 0.01) increased by germination but decreased by soaking and heat treatments. The increase in extractable protein may be resulted from the activity of the proteolytic enzymes in the sprouting seedlings. The results agree with the observed increase in nonprotein nitrogen during germination (Table 4).

The increase in the percentage of the insoluble protein complexes with carbohydrates and polyphenols, and the increase in protein denaturation have been found to reduce protein solubility\textsuperscript{[37,2]}.
Fig. 1a: Percent nitrogen extracted in water as a function of time of the total proteins from the flours of raw and processed bambara groundnut seed.

Values are means of triplicate samples. C: Control; S: Soaked seed; G2: 2-day old sprout; G4: 4-day old sprout; G6: 6-day old sprout; UNSCw: Unsoaked-cooked in water; UNSCs: Unsoaked-cooked in 2% NaCl; R: Roasted.

Fig. 1b: Percent nitrogen extracted in 1 M NaCl solution as a function of time of the total proteins from the flours of raw and processed bambara groundnut seed.

Values are means of triplicate samples. C: Control; S: Soaked seed; G2: 2-day old sprout; G4: 4-day old sprout; G6: 6-day old sprout; UNSCw: Unsoaked-cooked in water; UNSCs: Unsoaked-cooked in 2% NaCl; R: Roasted.

be responsible for the differences in solubility profiles.

Nitrogen Solubility Profiles in 1 M NaCl Solution:
Bambara groundnut seed proteins (control) extracted in 1 M NaCl solution at different pH values (Figure 1b), do not have a solubility minimum like that obtained in water (Figure 1a). Extractability of the control seed proteins in 1 M NaCl solution at pH 1-6 was increased significantly (p ≤ 0.01) on average ~ 3-fold that extracted in water. Salt solubility profiles of the soaked, germinated and heat treated samples showed significant (p ≤ 0.01) reduction in the amounts of the proteins extracted at the different pH levels compared to that of the control sample (Figure 1b). Sodium chloride can bring about charge differences on the protein, leading to change in solubility of the various protein fractions with different isoelectric regions.

Water and Fat Absorption Capacities: Water absorption capacity (WAC) and fat absorption capacity (FAC) of total proteins from the flours of raw (control) and processed bambara groundnut seed are presented in Table 5. Neither the WAC nor the FAC of the control flour (5.87 and 3.95 ml/g, respectively) was improved by applying domestic processing methods.

Foaming Capacity and Foam Stability: Table 6 shows that foaming capacity (FC) of bambara groundnut seed flour (59.00%) was decreased significantly (p ≤ 0.01) by soaking (12 hours), germination (2–6 days), roasting and cooking conditions. Heat treatments resulted in the highest reduction in foamability of the control flour. The increase in the fraction of the insoluble proteins (Table 4) may partially explain this reduction.

Foam stabilities (FS) of the control (C) and processed seed flours are shown in Table 6. The FS of the control in water has a value of 74.60%. The FS of the soaked seed (S) and 2-day old sprout (G2) did not vary significantly (p ≤ 0.01) from that of the control. The FS in water of 4-day old sprout (81.40%) and 6-day old sprout (88.90%) flours were significantly (p ≤ 0.01) higher than that of the control. The results agree with those found by Rahma in faba bean. Addition of 1 M NaCl decreased the foaming capacity of all the flours observed in water.

The decrease in foam volume, in water and 1 M NaCl, as a function of time is shown in Figure 2a and b. Control flour has stable foam. Compared to control, germination increased foam stability with time both in water and 1 M NaCl. Heat treated samples showed poor stability of the foams in the respective media. The differences in the foaming properties may result from the differences in protein solubility
Table 6: Functional properties of raw and processed bambara groundnut seed flour: in water and 1 M NaCl.

<table>
<thead>
<tr>
<th>Sample</th>
<th>FC%#</th>
<th>FS%#</th>
<th>EA</th>
<th>Absorbance at 500 nm</th>
<th>ES, seconds</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>Water</td>
<td>59.00 ± 3.41</td>
<td>74.60 ± 2.40</td>
<td>12.57 ± 0.23</td>
<td>1105 ± 36.80</td>
</tr>
<tr>
<td></td>
<td>1 M NaCl</td>
<td>41.00± 1.94</td>
<td>73.20 ± 4.10</td>
<td>13.94 ± 0.06</td>
<td>301 ± 24.00</td>
</tr>
<tr>
<td>S</td>
<td>Water</td>
<td>37.50± 2.12</td>
<td>73.00 ± 3.20</td>
<td>10.77 ± 0.52</td>
<td>1135 ± 7.07</td>
</tr>
<tr>
<td></td>
<td>1 M NaCl</td>
<td>35.00± 3.46</td>
<td>85.70 ± 2.90</td>
<td>12.80 ± 0.15</td>
<td>761 ± 26.90</td>
</tr>
<tr>
<td>G2</td>
<td>Water</td>
<td>39.50 ± 2.12</td>
<td>73.80 ± 2.22</td>
<td>13.40 ± 0.24</td>
<td>840 ± 17.00</td>
</tr>
<tr>
<td></td>
<td>1 M NaCl</td>
<td>32.80± 4.20</td>
<td>78.10 ± 3.30</td>
<td>12.41 ± 0.21</td>
<td>712 ± 19.80</td>
</tr>
<tr>
<td>G4</td>
<td>Water</td>
<td>43.00 ± 3.14</td>
<td>81.40 ± 94.30</td>
<td>13.90 ± 0.28</td>
<td>770 ± 14.24</td>
</tr>
<tr>
<td></td>
<td>1 M NaCl</td>
<td>34.16± 3.60</td>
<td>94.30 ± 3.36</td>
<td>12.63 ± 0.13</td>
<td>1558 ± 19.90</td>
</tr>
<tr>
<td>G6</td>
<td>Water</td>
<td>44.50 ± 2.34</td>
<td>88.90± 6.10</td>
<td>14.11 ± 0.16</td>
<td>720 ± 14.04</td>
</tr>
<tr>
<td></td>
<td>1 M NaCl</td>
<td>36.00± 2.52</td>
<td>88.90± 3.36</td>
<td>14.11 ± 0.16</td>
<td>1808 ± 48.00</td>
</tr>
<tr>
<td>SC</td>
<td>Water</td>
<td>12.00 ± 3.82</td>
<td>16.7 ± 1.27</td>
<td>21.56 ± 0.61</td>
<td>731 ± 26.80</td>
</tr>
<tr>
<td></td>
<td>1 M NaCl</td>
<td>2.40± 1.22</td>
<td>0.00</td>
<td>21.66 ± 0.56</td>
<td>140 ± 14.14</td>
</tr>
<tr>
<td>UNSCw</td>
<td>Water</td>
<td>13.50 ± 2.12</td>
<td>0.00</td>
<td>22.84 ± 0.20</td>
<td>851 ± 33.94</td>
</tr>
<tr>
<td></td>
<td>1 M NaCl</td>
<td>2.00± 1.8</td>
<td>0.00</td>
<td>21.74 ± 0.26</td>
<td>245 ± 9.27</td>
</tr>
<tr>
<td>UNSCs</td>
<td>Water</td>
<td>16.00 ± 2.40</td>
<td>0.00</td>
<td>23.00 ± 0.72</td>
<td>455 ± 19.80</td>
</tr>
<tr>
<td></td>
<td>1 M NaCl</td>
<td>3.00± 1.60</td>
<td>21.84 ± 0.44</td>
<td>131 ± 21.00</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>Water</td>
<td>12.22</td>
<td>11.90± 1.10</td>
<td>24.36 ± 0.39</td>
<td>320 ± 20.10</td>
</tr>
<tr>
<td></td>
<td>1 M NaCl</td>
<td>1.96± 1.80</td>
<td>23.02 ± 0.28</td>
<td>122 ± 17.2</td>
<td></td>
</tr>
</tbody>
</table>

*Means of triplicate samples ± SD. Means followed by different letters within a column are significantly different according to DMRT (p < 0.01).

**C: Control bambara groundnut seed; S: Soaked seeds; G2: 2-day old sprout; G4: 4-day old sprout; G6: 6-day old sprout; SC: Soaked-cooked seeds; UNSCw: Unsoaked-cooked in water; UNSCs: Unsoaked-cooked in 2% NaCl; R: Roasted.


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**Fig. 2a:** Percent decrease in foam volume in water as a function of time of the total proteins from flour of raw and processed bambara groundnut seed.

(Table 3). Since these properties were found to be affected by salting-in and out of proteins[^10].

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**Fig. 2b:** Decrease in foam volume in 1 M NaCl solution as a function of time of the total proteins from flours of raw and processed bambara groundnut seed.

Values are means of triplicate samples. C: Control; S: Soaked seed; G2: 2-day old sprout; G4: 4-day old sprout; G6: 6-day old sprout; UNSCw: Unsoaked-cooked in water; UNSCs: Unsoaked-cooked in 2% NaCl; R: Roasted.

**Emulsification Activity and Emulsion Stability:** Emulsification activity (EA) of the control flour in...
Emulsion stability in water as a function of time of the total proteins from flours of raw and processed bambara groundnut seed.

Values are means of triplicate samples. C: Control; S: Soaked seed;
G2: 2-day old sprout; G4: 4-day old sprout; G6: 6-day old sprout; UNSCw:
Unsoaked-cooked in water; UNSCs: Unsoaked-cooked in 2% NaCl; R: Roasted.
The absorbance at 500 nm is measured (less than 1.0) and corrected for dilution
And plotted on the Y axis.

Emulsion stability (ES) of the control seed flour (C) was 1105s (Table 6). In water, ES of the germinated and heat treated samples was significantly lower than that of the control. Addition of 1 M NaCl to water decreased significantly (p ≤ 0.01) the ES in almost all the studied samples, except for the 4-day (G4) and 6-day (G6) old sprouts (1558s and 1808s, respectively).

ES as a function of time of samples in water and 1 M NaCl is shown in Figure 3a and b. In water, control, soaked, 4-day old sprout and 6-day old sprout are similar, indicating a gradual fall. In the presence of 1 M NaCl a significantly (p < 0.01) higher ES values for G4 (1558s) and G6 (1808s) were found. The changes in the nature of bambara groundnut seed proteins due to processing (Table 3 and 4) may be responsible for the changes in emulsification properties inherent in the control seed. The EA and ES were found correlated with hydrophobicity and solubility of the proteins.[31,36]

The functional behavior of the proteins could be affected by the presence of other constituents. Hence, caution should be considered in the interpretation of the results. The data clearly indicate that IVPD was improved by germination. Heat treatments were effective in improving IVSD, with cooking of the presoaked seeds being the most effective. Changes in protein conformation adversely affected the functional properties of the protein to a large extent. An understanding the variation in functional properties as a result of domestic processing methods would help in better utilization of these proteins in food systems. This would also help in deeper understanding the role of individual processing methods on the seed proteins present with other constituents.

REFERENCES


